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IDENTIFICATION AND CONTROL OF PETRO-
CHEMICAL POLLUTANTS INHIBITORY TO
ANAEROBIC PROCESSES

J. C. Hovious, et al

Union Carbide Corporation

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Identification and Control of Petrochemical Pollutants Inhibitory to Anaerobic Processes



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IDENTIFICATION AND CONTROL OF PETROCHEMICAL POLLUTANTS
INHIBITORY TO ANAEROBIC PROCESSES

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ABSTRACT

Identification studies were made on potentially inhibitory materials using a Warburg respirometer procedure and an unacclimated anaerobic biomass. Identified inhibitory materials and concentrations for a 50 percent decrease in activity were acrolein (20-50 mg/l), formaldehyde (50-100 mg/l), 2-ethyl-1-hexanol (500-1000 mg/l), methyl isobutyl ketone (100-300 mg/l), diethylamine (300-1000 mg/l), acrylonitrile (100 mg/l), 2-methyl-5-ethylpyridine (100 mg/l), ethylene dichloride (150-500 mg/l), ethyl acrylate (300-600 mg/l), and phenol (300-1000 mg/l). Inhibitory effects were more severe at high volatile acid concentrations.

Acclimation of anaerobic biomass to crotonaldehyde, phenol, ethyl acrylate, and sodium acrylate was studied in mixed digesters. An acclimated culture was developed for crotonaldehyde, phenol, and to some degree to ethyl acrylate. No acclimation was observed for sodium acrylate. Cultures acclimated to crotonaldehyde and ethyl acrylate were able to degrade the material while phenol was not degraded with acclimation but was no longer inhibitory.

Additional acclimation studies were made in continuously fed anaerobic filters. A filter was acclimated to a crotonaldehyde concentration of 600 mg/l as compared to the 50-100 mg/l inhibitory in Warburg studies. Treatment of formaldehyde, ethyl acrylate, phenol, and acrylonitrile indicated synergistic inhibitory effects. These mixed inhibitors were treated satisfactorily at low concentrations in two series anaerobic filters, however, increasing inhibitor concentrations resulted in failure of both filters. Actual waste streams treated in an anaerobic filter indicated that inhibition from crotonaldehyde could be avoided in a chemical manufacturing waste by dilution. Successful treatment of "hard" surfactant containing wastes was also noted. Other means of overcoming inhibition were discussed.

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SECTION I

CONCLUSIONS

1. A rapid, reproducible procedure using a Warburg respirometer was developed to assess and quantify the inhibitory effect of materials on unacclimated anaerobic microorganisms which metabolize acetate. This procedure provides lower threshold limits of material concentration for inhibition. Greater concentrations could possibly be tolerated by acclimated anaerobic organisms.
2. The α - β unsaturated aldehydes were particularly inhibitory to unacclimated anaerobic systems at low concentrations of 20-100 mg/l. Other materials which could result in inhibition problems were found to be 2-ethyl-1-hexanol, methyl isobutyl ketone, diethylamine, ethylenediamine, acrylonitrile, 2-methyl-5-ethylpyridine, phenol, ethylene dichloride, and ethyl acrylate.
3. Inhibition of gas production was found to be more severe in a system containing volatile acid concentrations greater than 500 mg/l as acetic. Inhibition also occurred at lower concentrations of test material under these conditions.
4. Introduction of test material at a gradually increasing level to continuously mixed batch-fed digesters resulted in acclimation and increased resistance to inhibition by crotonaldehyde, ethyl acrylate and phenol. Crotonaldehyde and ethyl acrylate were degraded completely in the acclimation digesters, while phenol was not. Similar acclimation experiments with sodium acrylate indicated no significant increase in resistance to inhibition.
5. Acclimation of anaerobic microorganisms in mixed digester cultures was found to be at best a difficult and long-term procedure with only moderate chances of success.
6. A continuously fed anaerobic, packed-bed filter was able to treat successfully a stream containing 600 mg/l crotonaldehyde at a 1.5 day detention time. Crotonaldehyde had been proved inhibitory to unacclimated microorganisms at 50 to 100 mg/l. The column failed at about a concentration range of 700 to 800 mg/l.

7. Treatment of four combined inhibitors in an anaerobic filter indicated a synergistic effect regarding the threshold limits for anaerobic inhibition.
8. Two series anaerobic filters were acceptable for treatment of the combined inhibitors at low concentrations. The initial column provided no oxygen-demand removal but modified the stream for treatment in a subsequent column. Increasing inhibitor concentrations resulted in complete inhibition of both columns.
9. Waste from a chemical production unit, containing a high concentration of crotonaldehyde, was biologically degraded in an anaerobic filter by a microbial culture acclimated by initial dilution and stepwise increases in waste concentration. A COD removal of 68 percent was observed.
10. A surfactant-containing petrochemical waste treated in an anaerobic filter exhibited complete removal of a "hard" nonionic surfactant at a concentration of 45 mg/l after acclimation. Higher concentrations resulted in leakage through the system.
11. An experimental system designed to quantify photosynthetic sulfide oxidation by bacteria of the family Thiorhodaceae was never able to maintain a significant population of these bacteria. The Chlorobacteriaceae which predominated did not provide significant sulfide oxidation.

SECTION II

RECOMMENDATIONS

It is recommended that:

1. Long-term continuously-fed reaction studies tailored to the expected treatment conditions be conducted for wastes having a mixture of anaerobic inhibitors or a system which will receive a continuous inhibitor dosage due to

- a) The synergistic effects of mixed inhibitors.
- b) The variation of inhibitory effect with system volatile acid levels.
- c) Bioacclimation which can be achieved with certain potentially inhibitory chemicals.

2. That the "activity ratio" procedure be utilized to determine approximate concentration limits for single material inhibition of unacclimated anaerobic systems. Such ratios would reflect the effects of "slug" doses on anaerobic systems.

3. That further study of photosynthetic, sulfur-oxidizing bacteria be conducted including the environmental conditions for optimal growth and the effects of petrochemical waste constituents on metabolism. Field studies are recommended since a significant population of these bacteria could not be maintained during laboratory studies conducted as part of this project.

4. Experimental results obtained by using acclimated sludge on a limited basis warrants further work in this area. It would be expected that highest activity ratios would be obtained by using an acclimated biota.

SECTION III

INTRODUCTION

Treatment of aqueous wastes from petrochemical manufacturing facilities has been found to be complicated components inhibitory to biological treatment systems at relatively low concentrations (1, 2). During an EPA funded anaerobic process development grant (12020-DIS), the use of high rate anaerobic systems was precluded due to inhibition problems. While considerable experimental effort has been spent on the behavior of specific synthetic organic materials in aerobic systems (3-8), virtually no work has been published on inhibition in anaerobic systems with the exceptions of volatile acids and inorganic materials (9). With the development of anaerobic processes specifically for petrochemical wastes (1, 2, 10), the need for inhibition data on specific materials is apparent. Such data are also needed for operation of anaerobic digesters receiving sludges from aerobic systems treating petrochemical wastes.

Inhibition of a microbiological process may result from a general effect on the environment of the process as a whole, or effects on the specific cells or enzymes within the process. As an example, addition of a strong acid in excess of system buffering capacity would result in inhibition due to low system pH while a mercury salt will combine with the enzymes containing sulfur in thiol groups (-SH) thus inhibiting activity. As the general environmental limits for anaerobic treatment are well defined and every attempt is made to control these in a treatment process, this study was centered on the effects of materials which would act on cellular constituents or enzyme systems.

Inhibition of a chemical on a cellular constituent may be the result of a reaction with some essential group of a protein or other molecule or adsorption at the cell surface. Such a reaction may result in conversion of a metabolically active substance to an inactive substance or a change in cell permeability. An example of reaction with an essential group of a protein would be the mercury-thiol bonding while surface active materials such as phenols are inhibitory due to interference with cellular permeability.

Enzyme inhibition results from competition between the inhibitor and substrate for attachment sites on the enzyme, an attraction of the inhibitor for a part of the enzyme, or destruction of an essential functional group of the enzyme. The relative effect of any inhibitory material on a microbial process may be expected to depend on the properties of the microbial growth. As an example, rapidly growing cells would be expected to be more susceptible to inhibitory materials than slower growing cells since a higher uptake and conversion of substrate is in progress. Also, microorganisms which grow in clusters or have slime layers would be expected to be less sensitive than dispersed organisms as less opportunity is available for inhibitor-organism contact.

Application of various inhibition theories to an anaerobic treatment process is complicated by the mixed biological cultures necessary within the process and the mixture commonly present in the waste. While a material may be inhibitory to one type of organism within a treatment system, there may well be others present which can degrade the material and thus overcome the inhibitory effects. Mixed chemicals could also have synergistic or antagonistic effects on the culture.

In order to develop a widely usable set of data, initial screening studies for inhibition were performed with a single material and an unacclimated, mixed anaerobic culture. Many materials which were found to be inhibitory in short-term screening studies were then subjected to acclimation procedures, either as the single compound or in a real waste stream to measure long-term effects in more realistic situations. Materials of particular interest were alpha-beta unsaturated carbonyl compounds, since these have been previously identified as inhibitory in aerobic system (5).

Once the inhibitory materials and related problem concentrations are known, procedures may be developed for control of the inhibitory effects. A classic example would be the control of heavy metal toxicity in digesters by the addition of sulfates which are reduced bacterially to sulfides, resulting in a non-toxic, heavy metal-sulfide precipitate.

SECTION IV

IDENTIFICATION OF INHIBITORY CHEMICALS

Initial Screening Tests

In order to screen the effects of a number of materials on anaerobic processes, a method was needed which would provide direct, reproducible inhibition data with a minimum effort for each material tested.

Metabolic parameters such as cell growth, organic reduction, and gas uptake or production in anaerobic systems are normally utilized to monitor microorganism activity. The required methanogenic phase of a non-photosynthetic anaerobic process is generally conceded to be both the most sensitive and the rate-limiting step in anaerobic treatment, excluding systems based on nitrate or sulfate reduction. Careful measurement of gas produced in test units was, therefore, selected as the indicator of bacterial activity. Cell growth and organic reduction measurements were not considered suitable for the type of short-term, small-volume tests envisioned for this phase of the study.

The Warburg constant-volume respirometer was selected for use in screening tests due to its high sensitivity to gas pressure changes and the potential for rapid collection of data. Anaerobic Warburg procedures in the literature (11, 12), emphasize the importance of developing carefully controlled operating techniques. The necessity of an oxygen-free atmosphere over the anaerobic biomass during the system charging and throughout the test period presents operational problems which needed to be solved prior to conducting quantitative Warburg studies with the test chemicals.

Procedure

In initial qualitative tests, a Warburg respirometer fitted with 18 flasks and manometers was used to measure the effect of individual chemicals on the methanogenic bacteria by monitoring gas production. The flasks (140-ml volume) were charged initially with sufficient nitrogen-sparged distilled water so that when dilution water plus nutrients, domestic anaerobic sludge, and test chemicals were added, a final volume

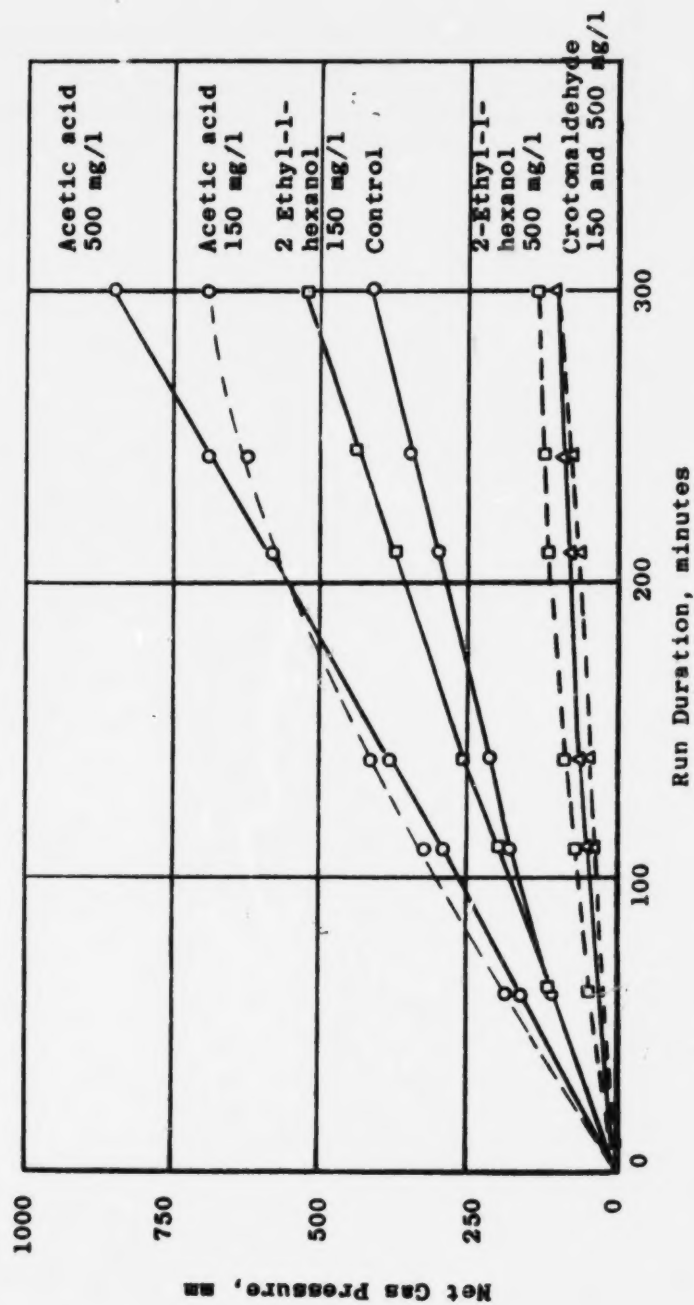
of 50 milliliters would result. The test chemical was added to give the desired final concentration (usually 150, 500 or 1000 mg/l). Aliquots of one-percent solutions of the water-soluble test chemicals were used, while insoluble chemicals were added via a micro-syringe technique. Twenty milliliters of nitrogen-sparged dilution water, containing one milliliter of buffer-nutrient solution per liter were then added, followed by 25 milliliters of undiluted anaerobic sludge conditioned at 35°C. All transfers were made under a nitrogen-gas blanket.

The flasks were then attached to the manometer and placed in the 35°C bath. The side arm of the flask was opened, and nitrogen was purged through the flask for 30 minutes. The nitrogen was turned off, the side arms closed and the shaker mechanism started. Gas pressure changes were very rapid during the first few minutes as equilibrium was reached between the dissolved gases in the sludge and the nitrogen atmosphere. After shaking 15 minutes, the manometers were leveled, and gas pressure was measured at desired intervals. Pressure readings were corrected for changes in atmospheric pressure and temperature by using a thermobarometer flask containing only distilled water.

Corrected gas pressure values were plotted against time for a period of 300 minutes to show the relative rate of gas production during this period.

Gas production of the control flask (seed biomass) would represent the cumulative production from methane fermentation, anaerobic respiration, and fermentation of carbohydrates and other materials in the domestic waste solids used as seed. Addition of a test chemical could affect the gas production in one of three ways - increased production, no change, or decreased production. An increased production would indicate that the test material was degradable by the anaerobic sludge, although not necessarily through methane fermentation. No change in gas production from the control indicates no net inhibition and/or degradation, while a decrease in gas production shows inhibition. The effect of some selected chemicals on the activity of domestic anaerobic sludge is shown in Figure 1.

FIGURE 1
EFFECT OF VARIOUS COMPOUNDS ON ANAEROBIC ACTIVITY OF
DOMESTIC SLUDGE



The activity of the test sludge system relative to that of the unfed sludge control was computed by dividing the millimeters of gas pressure built up by the sample by the millimeters of gas pressure built up by the control after 300 minutes. These data are summarized in Table 1.

TABLE 1
EFFECT OF VARIOUS COMPOUNDS TOWARD ANAEROBIC
ACTIVITY UNDER SUBSTRATE LIMITING CONDITIONS

Compound	Relative Activity with Respect to Control at Four Concentrations (mg/l) (2)			
	150	200	500	1000
t-Butanol	0.91	-	0.96	0.89
2-Ethyl-1-hexanol (3)	1.15	-	0.71	0.42
Crotonaldehyde (4)	0.23	-	-	0.23
Crotonaldehyde (4)	-	0.54	-	0.49
Methyl isobutyl ketone	1.05	-	1.05	1.03
Isophorone	1.13	-	1.12	0.81
Acrylonitrile	0.63	-	0.43	0.35
2-Methyl-5-ethyl pyridine (3)	-	1.20	-	0.65
N,N-Dimethylaniline	1.07	-	-	1.37
Phenol	1.06	-	1.15	1.02
Ethyl benzene (3)	0.96	-	0.96	1.10
Ethylene dichloride (3)	0.84	-	0.43	0.43
Ethyl acrylate	1.04	-	0.61	0.39
Dodecane (3)	1.04	-	1.10	1.27
Dextrose	-	1.98	-	2.30
Ethyl acetate	1.40	-	1.77	2.19
Ethylene glycol	1.46	-	1.65	1.70
Tetralin (3)	1.23	-	1.29	1.24
Sodium acetate	1.88	-	2.02	1.30
Mercuric chloride	-	-	0.27	-

(1) Initial studies performed under EPA Grant 12020-DIS, "Anaerobic Treatment of Synthetic Organic Wastes".

(2) The mm of gas pressure of a sample divided by mm gas pressure of control after 300 min; a value less than 1.00 indicates inhibition while a value greater than 1.00 indicates degradation.

(3) Compounds added with a micro-syringe because of solubility.

(4) Variable effect dependent upon system volatile acid concentration.

Methanogenic Inhibition

After completion of the initial screening effort, the need for a test more sensitive for detecting inhibition to methane bacteria was evident. A Warburg test procedure was developed similar to that used in the initial screening studies except that a degradable supplement of sodium acetate was added to ensure that the methanogenic bacteria would be functioning at a maximum rate in a non-food limited situation. This maximum metabolic rate would also be expected to result in greater sensitivity to inhibitory materials.

Lawrence and McCarty (13) have measured the rate of substrate utilization and the various constants for a formula similar to the Monod relationships for anaerobic methane fermentation:

$$dF/dt = \frac{kMS}{K_S + S}$$

where dF/dt = rate of waste utilization per unit volume of digester, mass/volume-time

M = microorganism concentration, mass/volume

S = waste concentration, mass/volume

k = maximum rate of waste utilization per unit weight of microorganisms occurring at high waste concentration, time⁻¹, $k = 8.1$ mg/mg-day for acetate substrate at 35°C, and

K_S = half velocity constant equal to the waste concentration when dF/dt is equal to one-half of the maximum rate, k , mass/volume, $K_S = 154$ mg/l for acetate substrate at 35°C

Calculation of substrate removal rate relative to the theoretical maximum at various substrate (acetate) concentrations is illustrated in Table 2.

TABLE 2
COMPUTATION OF RATE OF SUBSTRATE REMOVAL AT
VARIOUS SUBSTRATE CONCENTRATIONS

Substrate (a) Concentration, mg/l	$\frac{dF}{dt}$ M	$\left(\frac{\text{Substrate Removal Rate at Substrate Concentration}}{\text{Maximum Substrate Removal Rate}} \right) 100$
∞	8.1	100
154	4.05	50
500	6.19	76
1000	7.01	86
1500	7.34	91
2000	7.52	93

(a) Substrate considered to be acetate.

Assuming that gas production is directly related to substrate removal rate, the gas production rate would be similarly dependent on substrate concentration. A Warburg test was made to determine what level of added acetate would be required to yield a gas production rate independent of added feed in the test situation and type of seed sludge used. Results are presented in Table 3.

TABLE 3
RATE OF GAS PRODUCTION AT VARIOUS
SUPPLEMENTAL ACETATE LEVELS

Added Acetate Concentration, mg/l Ac ⁻	Rate of Gas Production, mm/hr
0	73
0	75
500	165
500	143
1000	142
1000	165

Warburg determination of duplicate
samples at 35°C

The data show an increase in gas production at both 500 and 1000 milligrams of added acetate per liter over the gas production of the unfed control, with no significant difference in production between the two acetate concentrations. Apparently with the sludge used in these particular experiments, an added acetate concentration of 500 mg/l in addition to the volatile acids contained in the digesting sludge was sufficient to guarantee a non-food-limited condition for gas production rate.

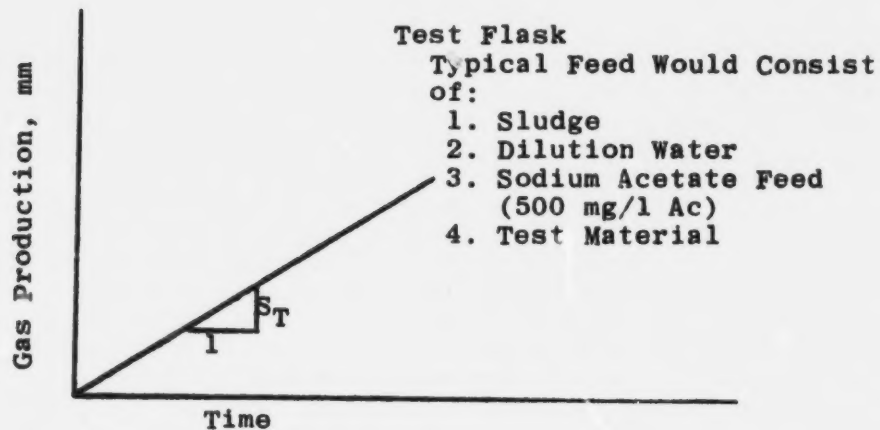
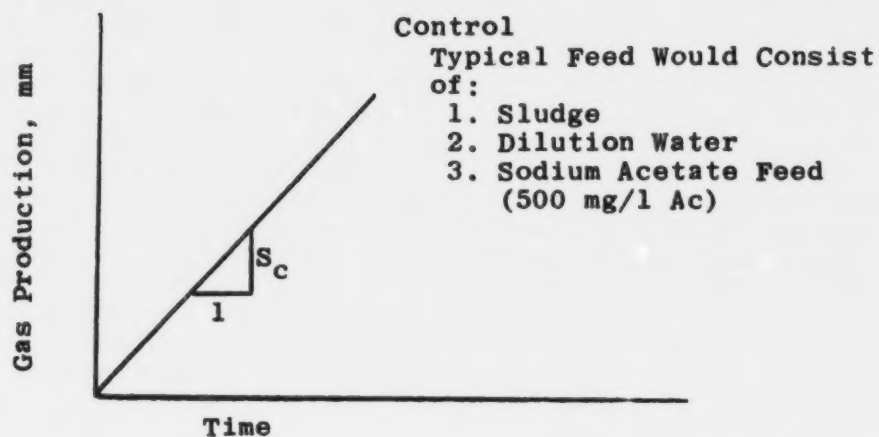
Experimental studies were set up in a fed-Warburg experiment in a similar fashion to the unfed experiments except 500 mg/l acetate (sodium salt used) was added to each Warburg flask. Also, the phosphate buffer addition was discontinued due to a possible reaction with bicarbonate to give an initial surge of carbon dioxide gas. The sludge itself was found to contain sufficient alkalinity without additional buffer.

In a non-inhibited, non-food-limited system the bacterial mass would be expected to operate at the maximum rate. In a system with a slowly changing bacterial mass, such as a culture of methanogenic bacteria with an acknowledged long generation time, the rate of gas production would then be expected to be linear with time over short-time intervals compared to the generation time. These linear plots were used to determine gas-production rates for both a fed-sludge control and a similar flask to which a test material was added. Slopes of the gas production with time were then compared to determine an activity ratio:

$$A = \frac{\text{gas production rate with test chemical}}{\text{gas production rate with fed sludge}}$$

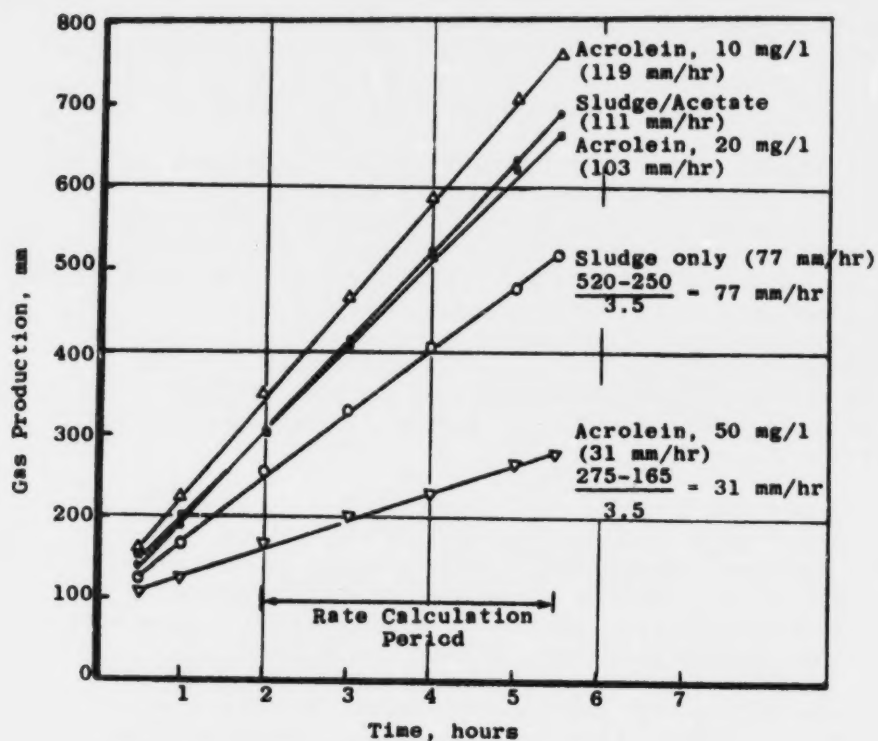
between the test material and sludge as shown in Figure 2. An activity ratio of 1.00 would indicate that the test material was not toxic to methane bacteria which ferment acetate. A ratio less than one would indicate inhibition of these bacteria while the magnitude of the ratio would act as an index of the amount of inhibition. In some special cases an activity greater than one could be observed which would indicate fermentation of a material through a pathway other than methane production from acetate - for example, fermentation of propionate to acetate or direct fermentation of a material such as methanol. Since the test biomass used was obtained from a domestic digester with no acclimation to petrochemical waste, the results should provide a minimum chemical concentration of the test material resulting in inhibition. Acclimation would result in less sensitivity to the test chemical or some greater concentration for the same inhibition. Results of a typical Warburg are presented in Figure 3.

FIGURE 2
FED-WARBURG DATA COLLECTION AND HANDLING



$$\text{Activity Ratio } A = S_T / S_c$$

FIGURE 3
TYPICAL WARBURG RESPIROMETER TEST DATA



Activity ratio calculation:

$$\frac{\text{Slope of Test Unit}}{\text{Slope of Acetate Control}} \quad \text{or} \quad \frac{31 \text{ mm/hr}}{111 \text{ mm/hr}} = 0.28 \text{ for } 50 \text{ mg/l acrolein concn.}$$

In order to compare the sensitivity of the method and the reliability of measuring changes in activity between various Warburg flasks, a study was made in which 17 flasks were filled with an identical fed sludge and the variation in linear gas production rates was measured. A standard deviation of 4.6 mm/hr from the mean of 27.4 mm/hr or 16 percent was noted in the reproducibility study. In addition, examination of the greatest number of data points collected for a given concentration of a particular chemical (300 mg/l phenol, 9 replicates) indicated a standard deviation of 27 percent (± 19) of the mean activity ratio of 0.69.

A listing of the types and structures of chemicals tested in the Warburg experiments is included in Table 4, while the results from the non-food limited Warburg studies are presented in Table 5. Rather than the three classifications available in the substrate-limiting studies, only two are generally available with the non-substrate-limiting tests, i.e., inhibitory or non-inhibitory. A compilation of the inhibition classifications obtained in the Warburg studies is provided in Table 6 for a comparison of the results of the two methods.

A gross comparison of the two experimental techniques indicates that when inhibition was observed in the substrate-limited experiments it was also observed in the non-substrate limited experiments (e.g., crotonaldehyde, acrylonitrile, and ethyl acrylate). However, in some cases inhibition was evidenced in the non-substrate-limited experiments where none was seen with limited substrate (methyl isobutyl ketone and phenol). Also, inhibition was generally more severe in the non-limited experiments (crotonaldehyde, acrylonitrile, 2-methyl-5-ethyl pyridine, and ethyl acrylate). The more rapid metabolic rate in the non-substrate-limited test provides one explanation for the greater sensitivity. Examination of the gas production occurring in the two types of tests and the resulting activity ratio provides another key to the more severe inhibition seen in the non-substrate-limited tests. The activity ratio (A) for any test may be expressed as

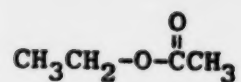
$$A = \frac{M_T + O_T}{M_C + O_C}$$

TABLE 4
CHEMICAL STRUCTURES EVALUATED IN WARBURG
INHIBITION STUDIES

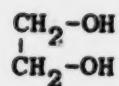
Chemical	Formula
<u>Alcohols:</u>	
n-Butanol(a)	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$
sec-Butanol(a)	$\begin{array}{c} \text{OH} \\ \\ \text{CH}_3\text{CHCH}_2\text{CH}_3 \end{array}$
t-Butanol(a,b)	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{C}-\text{OH} \\ \\ \text{CH}_3 \end{array}$
Allyl alcohol(a)	$\text{CH}_2=\text{CHCH}_2-\text{OH}$
2-Ethyl-1-hexanol(b)	$\begin{array}{c} \text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CHCH}_2-\text{OH} \\ \\ \text{CH}_2\text{CH}_3 \end{array}$
<u>Aldehydes:</u>	
Formaldehyde(a)	$\text{H}_2\text{C}-\text{O}$
Crotonaldehyde(a,b)	$\begin{array}{c} \text{CH}_3\text{C}-\text{CC}-\text{O} \\ \quad \\ \text{H} \quad \text{H} \end{array}$
Acrolein(a)	$\begin{array}{c} \text{CH}_2=\text{CHCH} \\ \\ \text{O} \end{array}$
<u>Ketones:</u>	
Acetone(a)	$\begin{array}{c} \text{O} \\ \\ \text{CH}_3\text{CCH}_3 \end{array}$
Methyl isobutyl ketone(a,b)	$\begin{array}{c} \text{O} \\ \\ \text{CH}_3\text{CHCH}_2\text{CCH}_3 \\ \\ \text{CH}_3 \end{array}$
Isophorone(b)	$\begin{array}{c} \text{O} \\ \\ \text{H}_2\text{C} \quad \text{CH} \\ \quad \quad \\ \text{CH}_3-\text{C} \quad \text{C}-\text{CH}_3 \\ \quad \quad \\ \text{CH}_3 \quad \text{H}_2 \end{array}$

TABLE 4 (CONT'D)

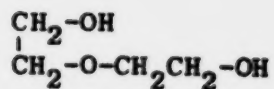
Ethyl acetate(b)



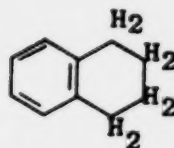
Ethylene glycol(a,b)



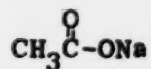
Diethylene glycol(a)



Tetralin(b)



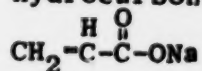
Sodium acetate(b)



Kerosene(a)

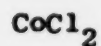
hydrocarbon 150-300°C

Sodium acrylate(a)



Inorganics

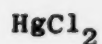
Cobalt chloride(a)



Hydrochloric acid(a)



Mercuric chloride(a)



(a) Tested in non-food-limited Warburg.

(b) Tested in Warburg with unfed sludge.

TABLE 4 (CONT'D)

Nitrogen Containing:

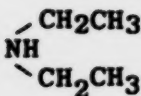
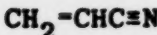
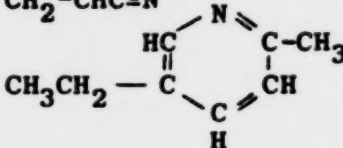
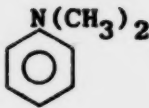
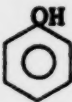
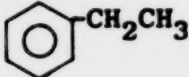
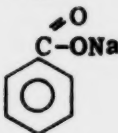
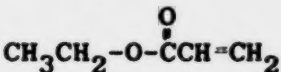
Diethylamine(a)	
Ethylene diamine(a)	$\text{NH}_2\text{-CH}_2\text{CH}_2\text{-NH}_2$
Acrylonitrile(a,b)	
2-Methyl-5-ethyl pyridine(a,b)	
N-N-dimethylaniline(b)	
<u>Aromatic:</u>	
Phenol(a,b)	
Ethyl benzene(b)	
Sodium benzoate(a)	
<u>Miscellaneous:</u>	
Chloroform(a)	CHCl_3
Ethylene dichloride(b)	$\text{Cl-CH}_2\text{CH}_2\text{-Cl}$
Ethyl acrylate(a,b)	
Dodecane(b)	$\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3$
Dextrose(a,b)	$\text{C}_6\text{H}_{12}\text{O}_6$

TABLE 5
INDIVIDUAL ANAEROBIC WARDING INHIBITION DATA
FOR NON-SUBSTRATE-LIMITING CONDITIONS

Chemical Tested	Inoculum Volume in Test Flask, ml	Gas Production, ml/g		Activity Relative to Inoculum Test Concentration (g)									
		(a)		(b)									
		Sediment Control	Test Chemical	10	20	50	100	200	500	1000	2000	5000	10000
n-Butanol	16,400	40	87	-	-	-	-	1.01	-	-	-	-	0.90
sec-Butanol	17,400	40	34	-	-	-	0.95	-	-	-	-	-	0.80
	16,700	40	87	-	-	-	0.81	0.86	-	-	-	-	0.74
t-Butanol	17,400	40	34	-	-	-	1.08	0.92	-	-	-	-	0.80
	16,700	40	87	-	-	-	0.95	0.86	-	-	-	-	0.80
Allyl alcohol	17,000	50	49	-	-	-	0.94	0.74	-	-	-	-	0.80
	14,400	25	104	-	-	-	0.86	0.99, 1.00	0.70	-	-	-	0.80
Formaldehyde	11,200	40	90	-	-	-	0.24	-	-	-	-	-	0.81
	15,500	-	81	1.1	-	0.37	0.34	-	0.34	-	-	-	-
	14,400	-	110	0.99	0.91	0.51	-	-	-	-	-	-	-
Crotonaldehyde	14,600	40	87	-	-	-	-	0.21	-	-	-	-	-
	9,600	34	180	-	-	-	0.3	0.11	0.11	-	-	-	-
	16,000	31	34	-	0.83	0.64	0.32	-	-	-	-	-	-
Acrolein	17,000	50	89	-	-	-	0.16	0.14	0.09	-	-	-	-
	17,500	-	81	1.00	-	-	0.18	-	0.06	-	-	-	-
	14,400	-	110	1.08	0.94	0.28	-	-	-	-	-	-	-
Acetone	14,400	26	72	-	-	-	1.01	1.01	0.99	-	-	-	1.00
Methyl isobutyl ketone	17,000	55	67	-	-	-	0.83	0.81	-	-	-	-	0.31
	14,400	26	72	-	1.00	1.06	-	-	-	-	-	-	-
Diethylamine	11,000	47	84	-	-	-	1.05	1.04	-	-	-	-	0.2
	22,500	111	224	-	-	-	0.88	0.69	-	-	-	-	-
Ethylene diamine	14,400	26	72	-	1.01	-	0.71	0.21	-	-	-	-	-
Acrylonitrile	16,200	40	87	-	-	-	0.62	0.50	0.40	-	-	-	0.26
N-Methyl-2-methylpyrrolidone	17,000	55	67	-	-	-	0.50	0.37	-	-	-	-	0.29
	14,400	26	72	-	1.00	1.00	-	-	-	-	-	-	-
Phenol	9,000	74	160	-	-	-	0.8	0.4	-	-	-	-	0.16
	6,400	135	164	-	-	-	0.7	0.2	-	-	-	-	0.21
	2,000	44	100	-	-	-	0.6	-	-	-	-	-	-
	4,000	11	91	-	-	-	0.43	0.28	-	-	-	-	-
	1,000	-	72	-	-	-	0.52	0.40	-	-	-	-	0.27
	1,400	34	72	-	-	-	0.9	0.76	-	-	-	-	0.12
	2,500	50	94	-	-	-	-	0.75	-	-	-	-	0.12
	11,200	74	116	-	-	-	1.64	-	0.88	-	-	-	0.12
	14,400	71	104	-	-	-	0.92	-	0.86, 0.88	-	-	-	0.35, 0.33
Sodium benzoate	12,600	40	54	-	-	-	1.09	-	1.10	-	-	-	-
Chloroform	14,600	-	72	-	-	-	0.31	-	0.35	-	-	-	-
	10,900	97	149	-	-	-	-	-	-	-	-	-	0.17
	13,400	135	203	-	-	-	0.18	-	0.18	-	-	-	0.19
Ethyl acetate	9,000	74	160	-	-	-	0.70	0.60	-	0.35	-	-	-
	24,400	70	116	-	-	-	0.93	0.88	-	-	-	-	0.21
	12,200	26	49	-	-	-	0.56	0.32	-	-	-	-	-
	1,000	34	72	-	-	-	0.80	0.76	-	0.35	-	-	-
	2,500	50	94	-	-	-	-	0.6	-	0.25	-	-	-
Sodium acetate	15,400	40	90	-	-	-	0.74	-	0.54	-	-	-	-
	15,500	-	81	1.3	1.0	1.0	-	-	-	-	-	-	-
	14,400	-	110	1.08	1.03	1.06	-	-	-	-	-	-	-
Dextrose	17,000	55	67	-	-	-	-	0.99	-	-	-	-	1.17
	16,200	-	88	-	-	-	-	1.25	-	-	-	-	1.40
Ethylene glycol	9,000	74	160	-	-	-	-	1.3	-	-	1.3	-	-
	11,200	70	116	-	-	-	-	1.12	-	-	1.07	-	-
Diethylene glycol	14,400	26	72	-	0.84	0.97	-	0.88	-	-	-	-	0.86
Benzene	17,000	55	69	-	-	-	0.96	0.96	0.91	-	-	-	-
Cobalt chloride	15,500	-	81	-	-	-	1.08	-	1.16	-	-	-	1.22
Hydrochloric acid	10,900	97	149	-	-	-	-	-	-	-	-	-	0.10(d)
Barium chloride	10,900	97	149	-	-	-	-	-	-	-	-	-	0.17

(a) Gas production as indicator of sludge activity is measured by comparing sludge alone against sludge or biomass fed 500 mg/l of acetate added (as sodium salt).

(b) Value presented is the ratio of the rate of gas production of the test chemical flask to the acetate-containing, reference flask.

(c) This value is probably low and failed to indicate the level of inhibition expected at these low solids concentration.

(d) The pH of the sludge/acetate mixture was adjusted to 2 with hydrochloric acid for a period of 15 minutes prior to neutralizing with sodium hydroxide and initiating the gas monitoring portion of the test.

TABLE 6
SUMMARY OF EFFECTS OF TESTED MATERIALS
ON ANAEROBIC ACTIVITY

<u>Chemical</u>	<u>Substrate Limited Experiments (1)</u>	<u>Non-Substrate Limited Experiments (2)</u>
n-Butanol	NT(3)	No inhibition
sec-Butanol	NT	Slight inhibition(7)
t-Butanol	No inhibition(4)	Slight inhibition
Allyl alcohol	NT	Slight inhibition
2-Ethyl-1-hexanol	Inhibition(6)	NT
Formaldehyde	NT	Inhibition
Crotonaldehyde	Inhibition	Inhibition
Acrolein	NT	Inhibition
Acetone	NT	No inhibition
Methyl isobutyl ketone	No inhibition	Inhibition
Isophorone	No inhibition	NT
Diethylamine	NT	Inhibition
Ethylenediamine	NT	Inhibition
Acrylonitrile	NT	Inhibition
2-Methyl-5-ethyl- pyridine	NT	Inhibition
N,N-dimethylaniline	No inhibition	NT

TABLE 6 (CONT'D)

<u>Chemical</u>	<u>Substrate Limited Experiments</u>	<u>Non-Substrate Limited Experiments</u>
Phenol	No inhibition	Inhibition
Ethyl benzene	"	NT
Sodium benzoate	NT	No inhibition
Ethylene dichloride	Inhibition	NT
Ethyl acrylate	Inhibition	Inhibition
Dodecane	No inhibition	NT
Dextrose	Increased activity(5)	No inhibition
Ethyl acetate	Increased activity	NT
Ethylene glycol	Increased activity	No inhibition
Diethylene glycol	NT	Slight inhibition
Tetralin	No inhibition	NT
Kerosene	NT	No inhibition
Sodium acrylate	NT	Inhibition
Cobalt chloride	NT	No inhibition

- (1) From Table 1, substrate level same as unfed control.
- (2) From Table 5, substrate level 500 mg/l acetate.
- (3) Not tested.
- (4) No inhibition at maximum test concentration tested (1000 mg/l).
- (5) Increased activity over that observed in unfed sludge control.
- (6) Inhibition evidenced, at least at 1000 mg/l level of test chemical.
- (7) Slight change in activity ratio at maximum concentration tested.

- where M = methanogenic gas production from bacteria capable of fermenting or anaerobically respiring acetate
- O = other gas production, fermentation of higher volatile acids, direct fermentation of simple compounds, or gas production during breakdown to volatile acids
- T = conditions in test flask
- C = condition in control flask

In the non-limited substrate experiments M would be a maximum due to the conditions of the test and would, therefore, be expected to be of more significance in the ratio than in the limited-substrate tests. Therefore, a change in " M_T " would result in a greater effect on "A" in an unlimited system than would the same change in a food-limited system. A greater effect on the measured activity ratio would, therefore, result.

Also, in the tests in which substrate was limited the addition of food (in the form of a test chemical) could cause an increase in " O_T " or conceivably in " M_T " by an increase in rate of gas production in those bacteria remaining viable. In either case, the non-limited substrate experiments would provide a more sensitive and accurate relationship for inhibition to bacteria which ferment acetate.

Results of the activity-ratio vs concentration experiment for those chemicals which showed significant inhibition in the more sensitive, non-substrate limited test procedure are illustrated in Figures 4 through 14. In addition, the effect of some materials found inhibitory in substrate limited experiments is illustrated in Figures 15 and 16. The relative amount of inhibition (decrease of activity ratio) may be compared with that experienced in experiments where an attempt was made to inhibit anaerobic activity by adding known toxicants (chloroform, mercuric chloride, and acidification with hydrochloric acid). In these tests, a residual activity ratio of 0.17 to 0.19 was measured at 1000 mg/l dosages of the chloroform and mercuric chloride or acidification to

FIGURE 4
EFFECT OF ACROLEIN ON ANAEROBIC ACTIVITY

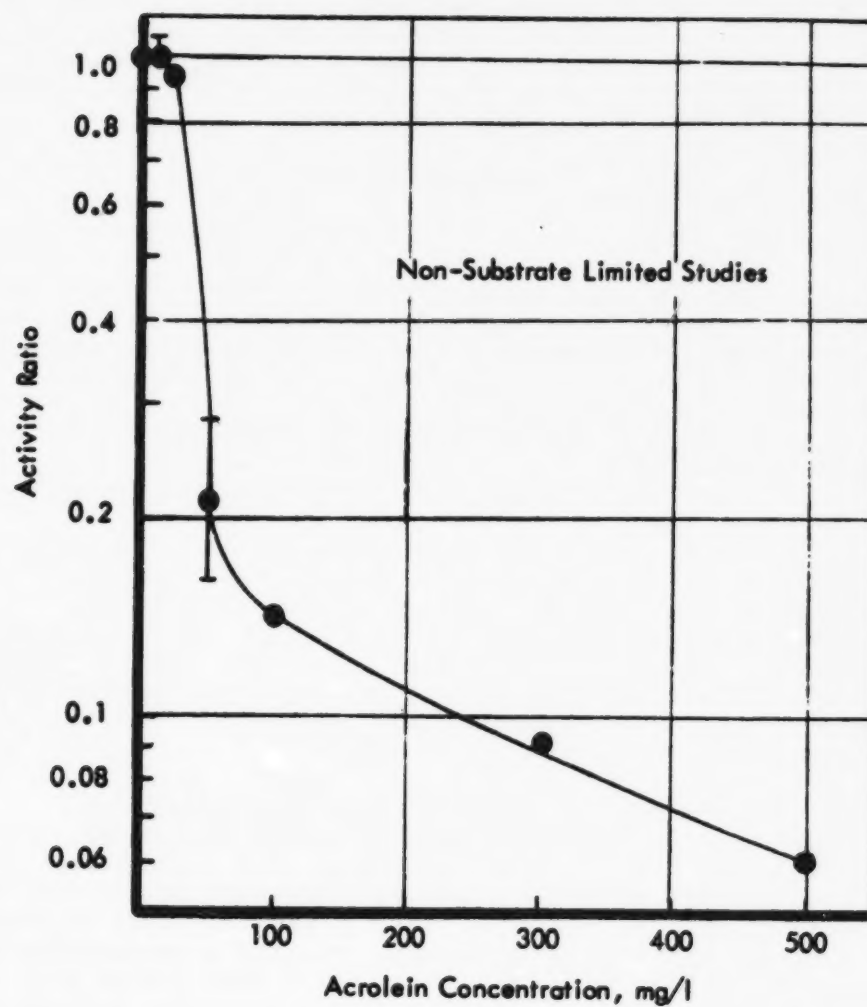


FIGURE 5
EFFECT OF CROTONALDEHYDE ON ANAEROBIC ACTIVITY

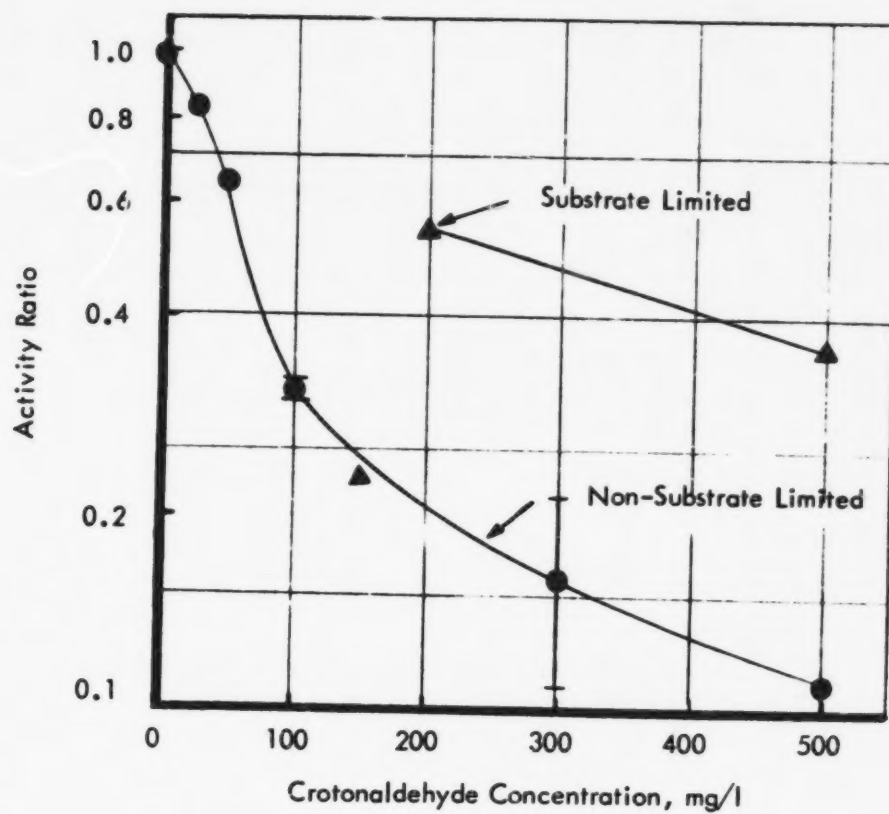


FIGURE 6
EFFECT OF FORMALDEHYDE ON ANAEROBIC ACTIVITY

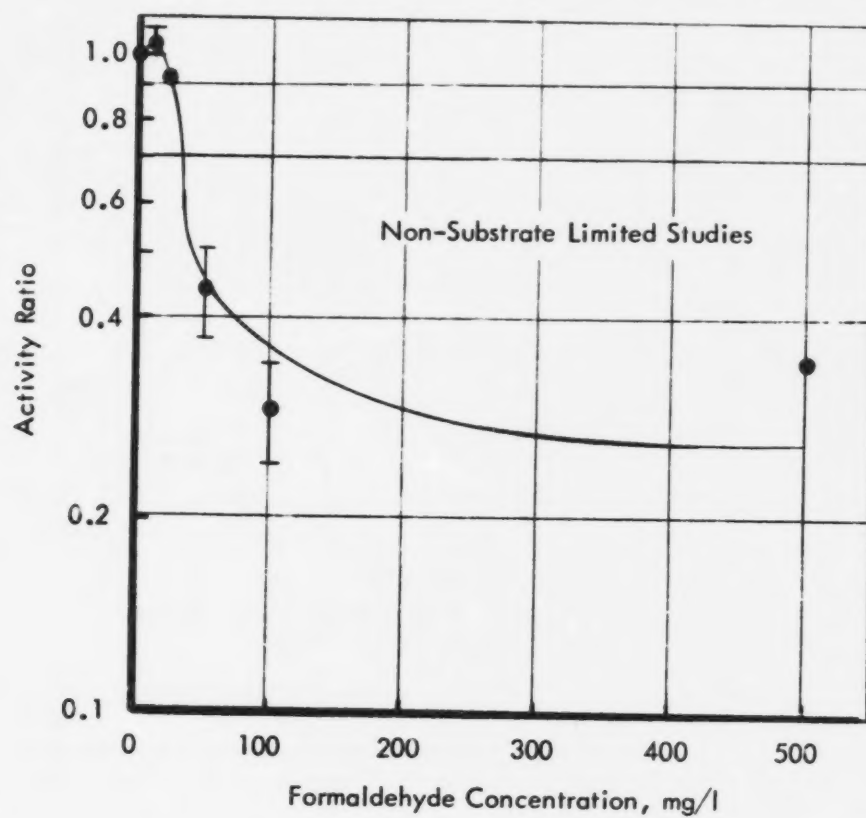


FIGURE 7
EFFECT OF METHYL ISOBUTYL KETONE ON ANAEROBIC ACTIVITY

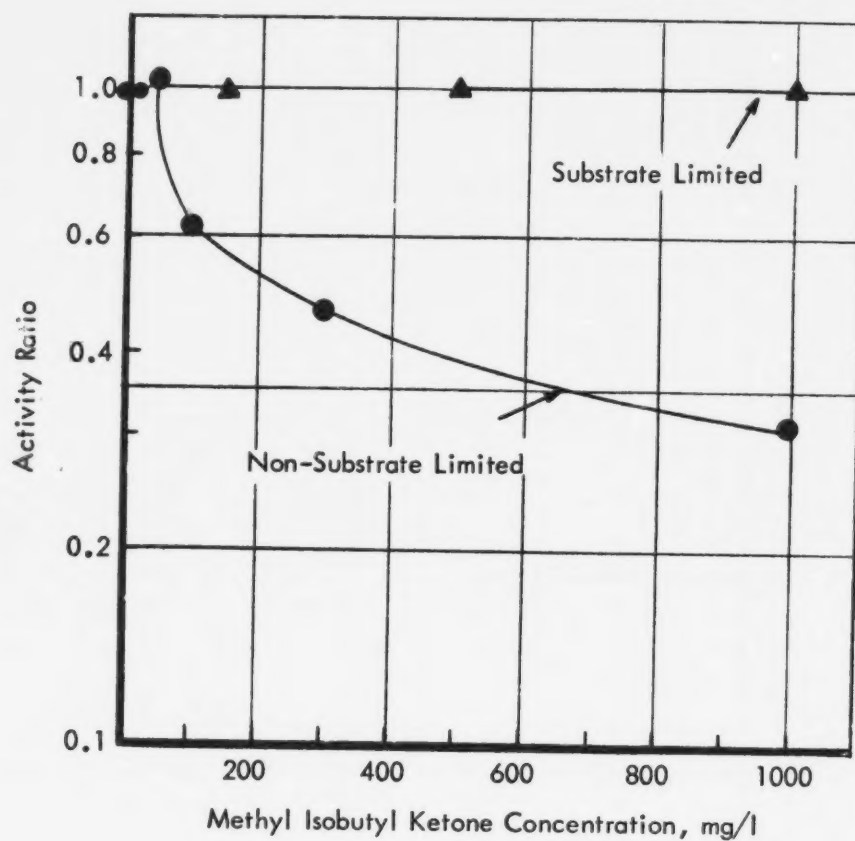


FIGURE 8

EFFECT OF ETHYLENEDIAMINE ON ANAEROBIC ACTIVITY

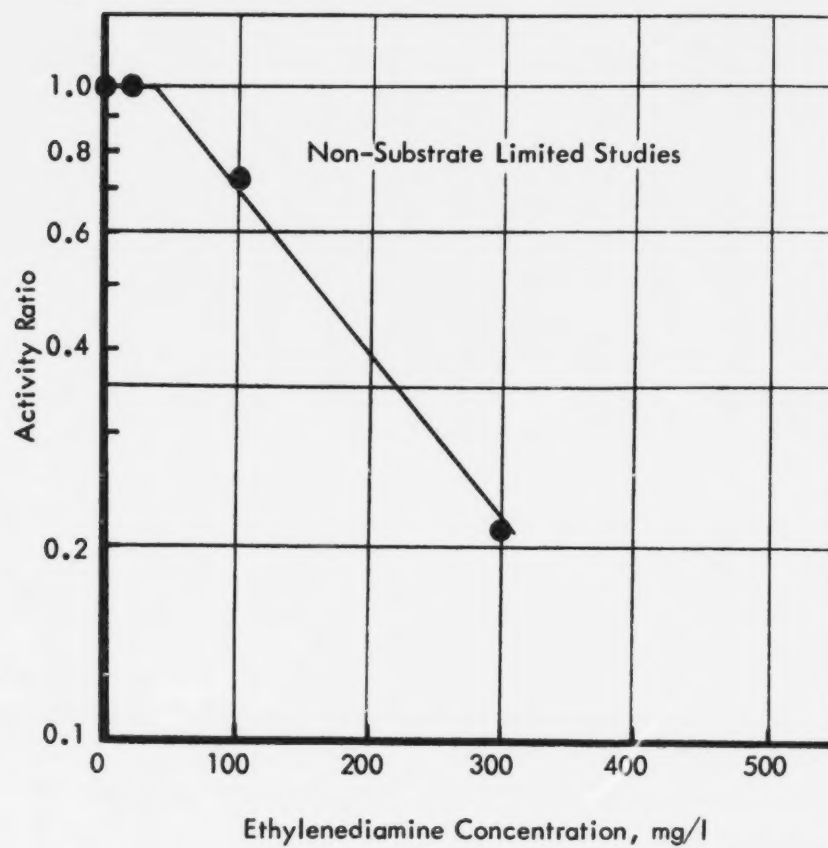


FIGURE 9

EFFECT OF DIETHYLAMINE ON ANAEROBIC ACTIVITY

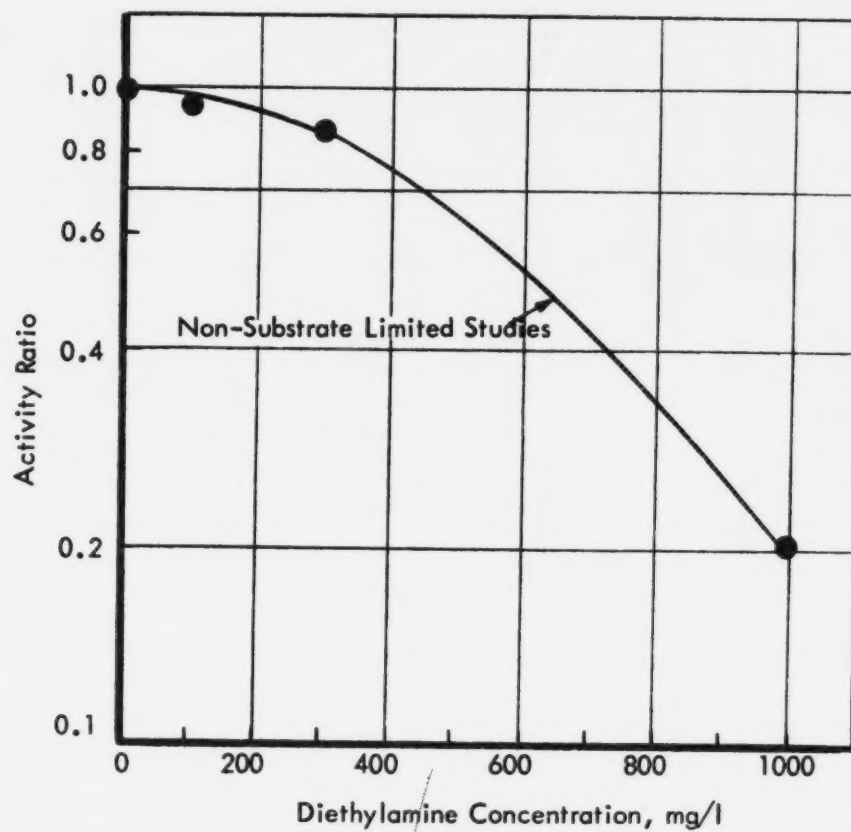


FIGURE 10
EFFECT OF ACRYLONITRILE ON ANAEROBIC ACTIVITY

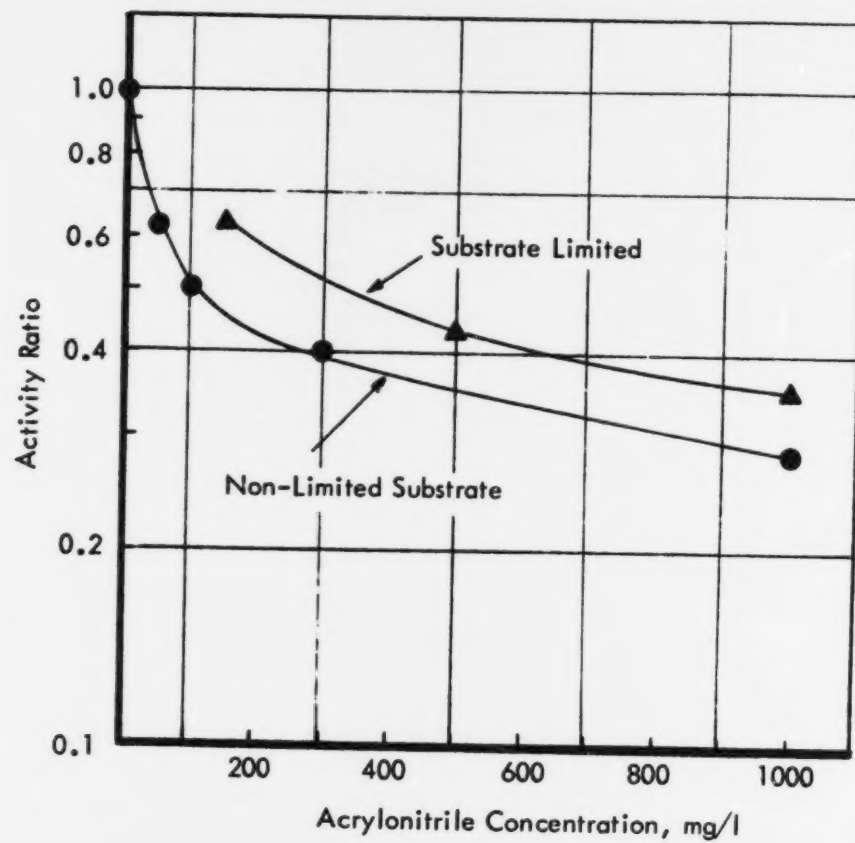


FIGURE 11
EFFECT OF 2-METHYL-5-ETHYLPYRIDINE ON ANAEROBIC ACTIVITY

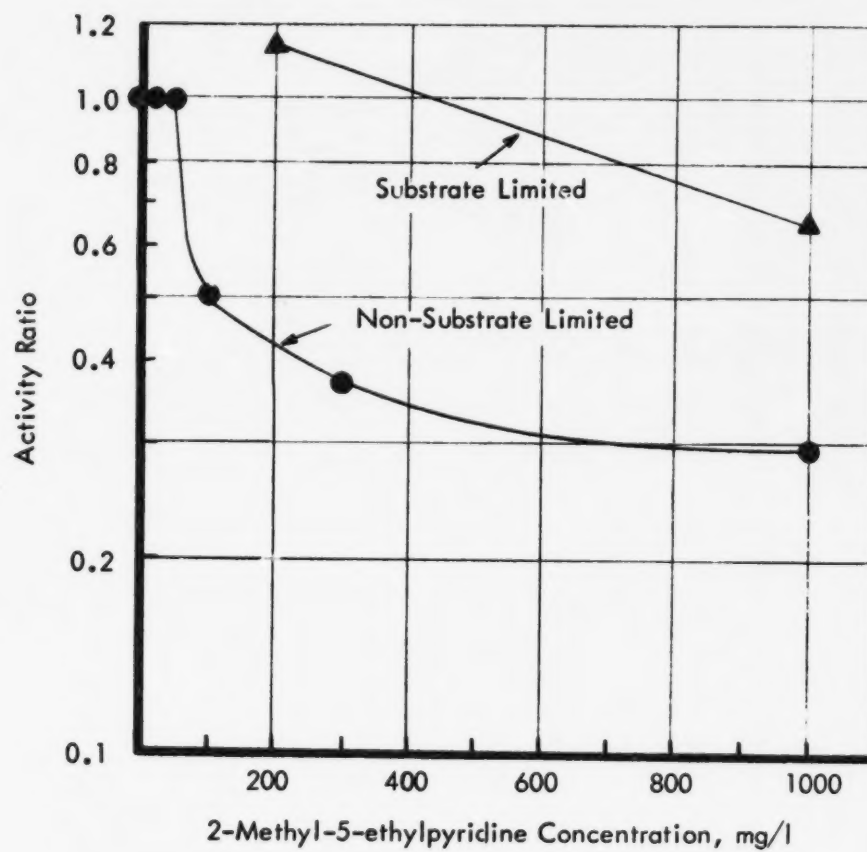


FIGURE 12
EFFECT OF PHENOL ON ANAEROBIC ACTIVITY

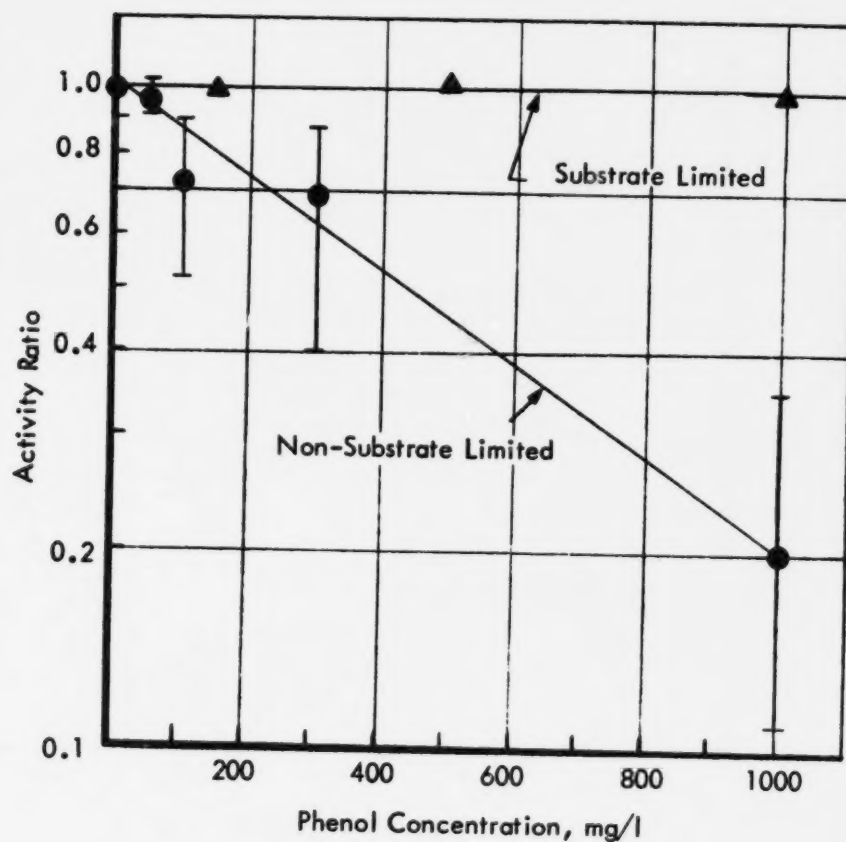


FIGURE 13
EFFECT OF SODIUM ACRYLATE ON ANAEROBIC ACTIVITY

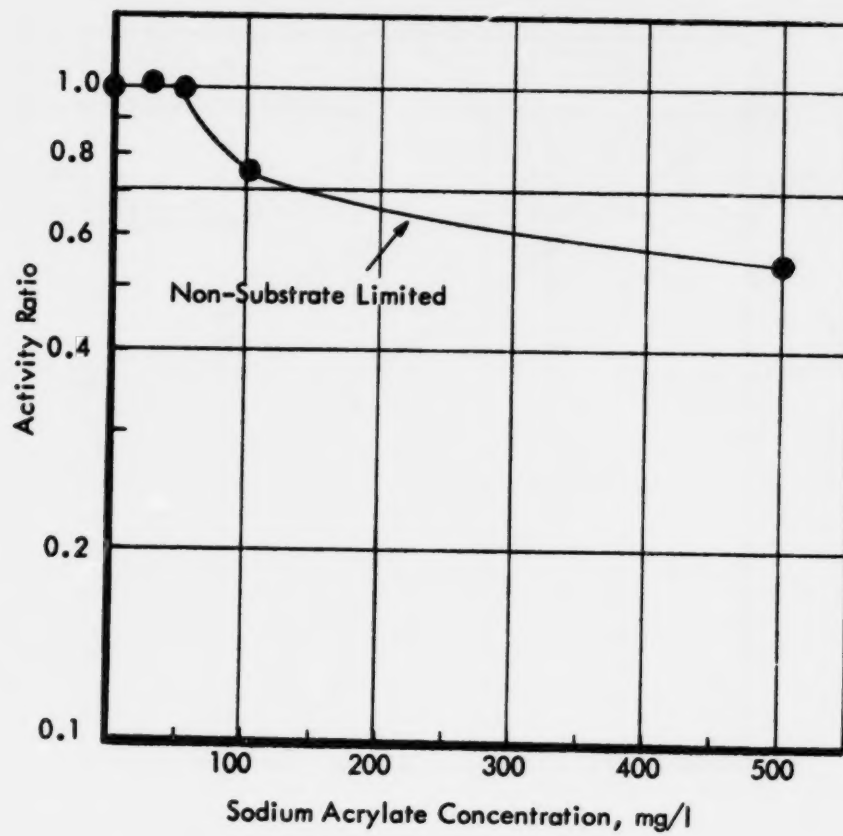


FIGURE 14
EFFECT OF ETHYL ACRYLATE ON ANAEROBIC ACTIVITY

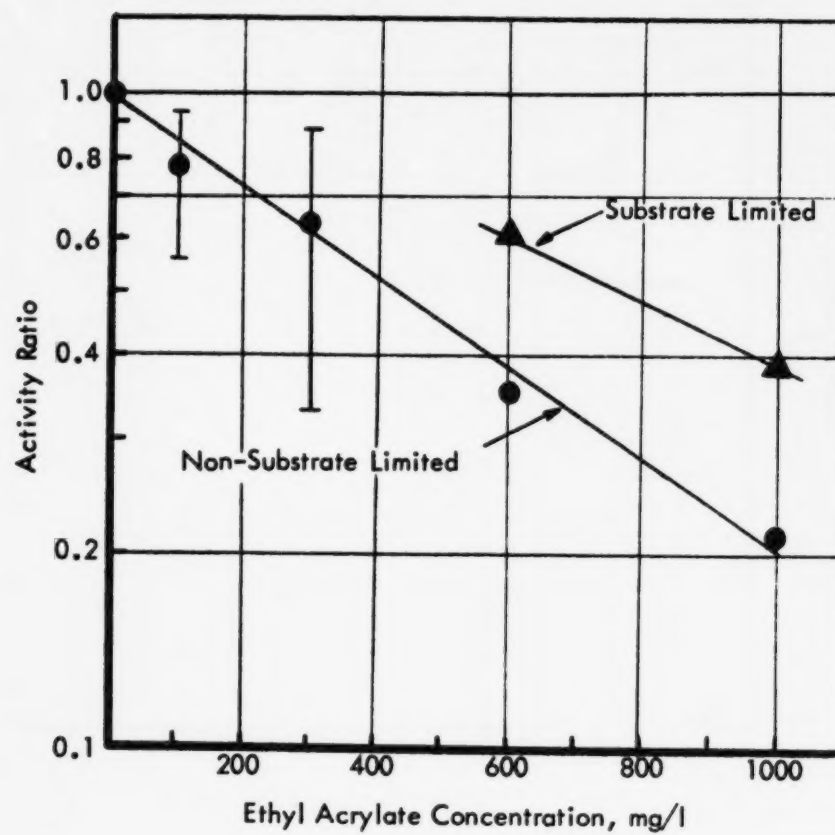


FIGURE 15
EFFECT OF 2-ETHYL-1-HEXANOL ON ANAEROBIC ACTIVITY

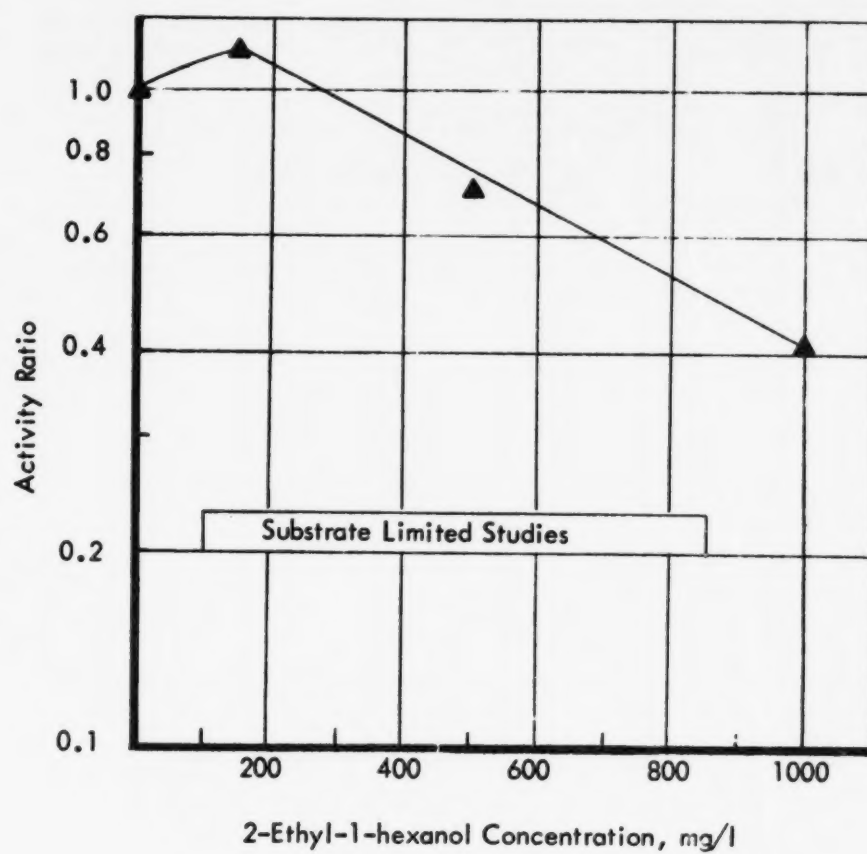
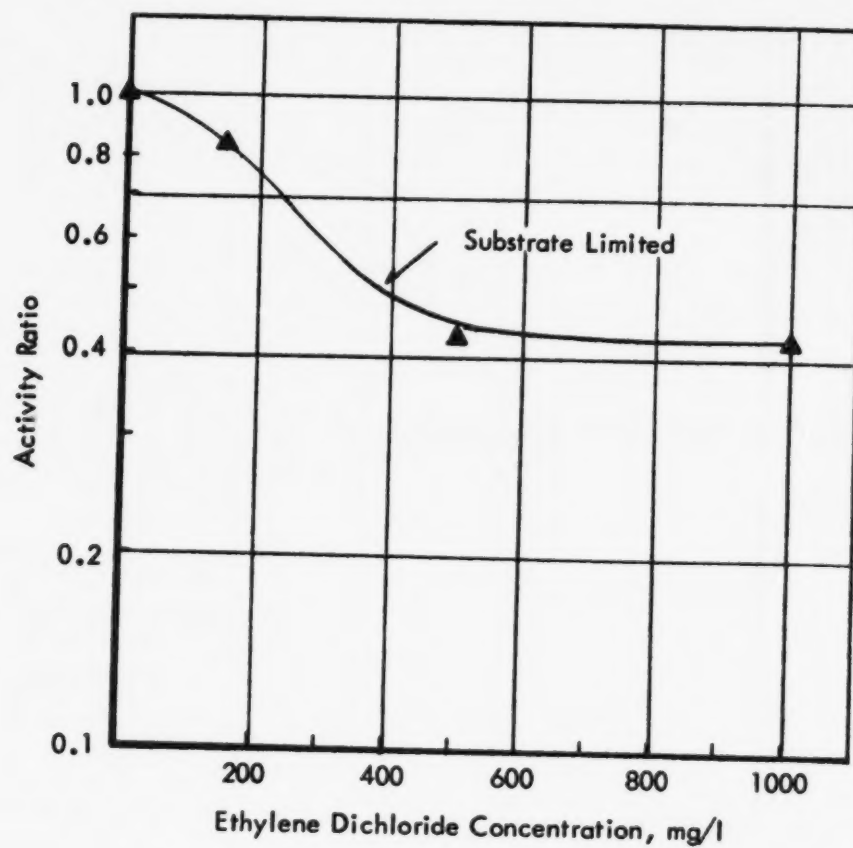


FIGURE 16
EFFECT OF ETHYLENE DICHLORIDE ON ANAEROBIC ACTIVITY



pH 2 in the non-substrate-limited tests. A residual ratio of 0.27 was measured at a mercuric chloride dosage of 500 mg/l in the food-limited tests.

All of the aldehydes tested (formaldehyde, crotonaldehyde, and acrolein) exhibited severe effects; acrolein (Figure 4) displayed the most adverse effect (inhibitions below that observed in the intentionally exhibited systems) at concentrations of 100 mg/l followed by crotonaldehyde (Figure 5, below killed-system activity at 300 mg/l) and formaldehyde (Figure 6). All compounds were a definite problem at the 50 mg/l concentration level. In the case of crotonaldehyde, a greater inhibition was observed in the non-substrate-limited study, which could possibly be explained as previously described.

In the studies with methyl isobutyl ketone (Figure 7), no inhibition was noted in the substrate-limited experiments. Although an initial rise in activity was noted at a 50 mg/l dosage level, a significant inhibition was apparent at 100 mg/l and above in non-limited systems. Inhibition was found to increase with concentration but was less than experienced in the intentionally inhibited systems indicating some residual activity.

Experiments were conducted with five nitrogen-containing compounds -- diethylamine, ethylenediamine, acrylonitrile, 2-methyl-5-ethyl pyridine, and N,N-dimethylaniline. Ethylenediamine (Figure 8) was found to be severely inhibitory ($A = 0.21$) at 300 mg/l with a slight decrease in activity at 100 mg/l in non-substrate limited experiments. Diethylamine (Figure 9) was less inhibitory than the ethylenediamine, an activity of 0.20 was measured at the 1000 mg/l concentration. The 0.2 activity ratio is comparable to that observed in the system which were intentionally inhibited.

Acrylonitrile is one of the few synthetic organic materials which has been examined previously (14) in anaerobic systems. Acrylonitrile additions of 50 and 100 mg/l resulted in a marked decrease in activity to a ratio of 0.5 at the 100 mg/l level in non-substrate-limited tests (Figure 10). A further decrease in activity was noted up to 1000 mg/l, the highest concentration tested. Inhibition was found

in substrate-limited experiments. As noted with other materials, this inhibition was not so severe as in the experiments with excess substrate. Residual activity was noted since in no case was the inhibition observed as severe as those systems which were intentionally inhibited.

Experiments with 2-methyl-5-ethylpyridine indicated no toxicity up to 50 mg/l concentration, and inhibition at 100 mg/l and above in non-substrate limited tests (Figure 11). With substrate limiting an apparent degradation of the material was noted at 200 mg/l with a slight inhibition at 1000 mg/l.

Phenol (Figure 12) was tested for inhibition as an example of an aromatic compound which is known to be inhibitory to unacclimated aerobic systems. In non-substrate-limited experiments phenol was increasingly inhibitory at concentrations up to 1000 mg/l, the highest level tested. No inhibition and no additional gas production was noted over the range of 0 to 1000 mg/l in substrate-limited tests.

Two acrylates, sodium acrylate (Figure 13) and ethyl acrylate (Figure 14) were found to be inhibitory. Sodium acrylate was slightly inhibitory on non-substrate-limited experiments at concentrations of 100 mg/l and above while ethyl acrylate was more inhibitory at higher concentrations. Ethyl acrylate, as mentioned previously for other compounds, was more inhibitory in non-substrate-limited experiments.

Two materials (2-ethyl-1-hexanol and ethylene dichloride) were found inhibitory in substrate limited studies. Activity for 2-ethyl-1-hexanol (Figure 15) exhibited a classical response to increases in 2-ethyl-1-hexanol concentration; a small amount stimulated activity while larger concentrations were inhibitory. Ethylene dichloride was also tested in a substrate-limiting experiment and found to be inhibitory. The materials were not tested in unlimited substrate experiments, but based on data for other materials, should be considered inhibitory.

Discussion

Examination of the inhibition data from the screening-type test yields several notable points both for overcoming the effects of inhibitory material and for application of these data to operating systems. The α - β unsaturated compounds were found to be inhibitory to anaerobic systems; the aldehydes producing the most severe problems. Inhibition in all cases was most pronounced in systems which were non-substrate limited. Such a situation would be comparable to a highly-loaded anaerobic treatment unit or a treatment unit with a high volatile acid content. A decrease in inhibition was noted with a lower volatile acid content.

Experimental data for the screening studies was obtained using only an active methane-producing, unacclimated, domestic digester sludge. In many cases acclimation of methane-forming or other types of bacteria presumably could develop a greater resistance to the particular compound. A summary of problem concentrations for the various tests is provided in Table 7.

TABLE 7

IDENTIFIED PROBLEM CONCENTRATIONS OF TESTED MATERIALS^(a)

<u>Chemical</u>	<u>Problem Concentration</u>	
	<u>Substrate Limiting</u>	<u>Non-Substrate Limiting</u>
n-Butanol	-	>1000 mg/l
sec-Butanol	-	>1000 mg/l
t-Butanol	>1000 mg/l	>1000 mg/l
Allyl alcohol	-	>1000 mg/l
2-Ethyl-1-hexanol	500-1000 mg/l	-
Formaldehyde	-	50-100 mg/l
Crotonaldehyde	~ 200 mg/l	50-100 mg/l
Acrolein	-	20-50 mg/l
Acetone	-	>1000 mg/l
Methyl isobutyl ketone	>1000 mg/l	100-300 mg/l
Isophorone	>1000 mg/l	-
Diethylamine	-	300-1000 mg/l
Ethylene diamine	-	100-300 mg/l
Acrylonitrile	150-500 mg/l	100 mg/l
2-Methyl-5-ethylpyridine	>1000 mg/l	100 mg/l
N,N-dimethylaniline	>1000 mg/l	-

TABLE 7 (CONT'D)

Phenol	>1000 mg/l	300-1000 mg/l (~ 400)
Ethyl benzene	>1000 mg/l	-
Sodium benzoate	-	>300 mg/l
Ethylene dichloride	150-500 mg/l	-
Ethyl acrylate	600-1000 mg/l	300-600 mg/l
Sodium acrylate	-	>500 mg/l
Dodecane	>1000 mg/l	-
Dextrose	>1000 mg/l	>1000 mg/l
Ethyl acetate	>1000 mg/l	-
Ethylene glycol	>1000 mg/l	>900 mg/l
Diethylene glycol	-	>1000 mg/l
Tetralin	>1000 mg/l	-
Kerosene	-	>500 mg/l
Cobalt chloride	-	>1000 mg/l

(a) Compound was arbitrarily classified as a problem when the activity ratio was less than 0.5. The 0.5 level was considered to be a decrease of more than 50% of the total gas production.

SECTION V

CONTROL OF INHIBITORY MATERIALS

Once problem compounds and associated inhibitory concentrations of these materials are identified, it becomes necessary to avert the potential inhibition if anaerobic treatment is to remain a viable alternative. Since the anaerobic process undoubtedly would be employed as a pretreatment step (the anaerobic effluent is generally not suited for discharge to the environment), a bypass of anaerobic pretreatment for a problem stream could be utilized.

Another potential control mechanism could be dilution of the problem waste with other waste streams to maintain a concentration below that found to be inhibitory. Dilution with clean water would be unfeasible as the economics of anaerobic treatment are based on treatment of a concentrated waste and a low-flow volume is desirable. Either effluent recycle or other wastes could be used for dilution.

If the inhibitory material occurred in batch discharges, impounding and a slow release to treatment at a concentration less than inhibitory could effectively avoid problems.

Design of a lower rate anaerobic system such as the anaerobic lagoon utilized in previous studies (1, 2, 10) would also be effective in combating inhibition due to the greater inhibition observed in a highly loaded, rapidly growing system.

Perhaps the most effective means of overcoming inhibition with a steady feed of inhibitory material would be through bacterial acclimation to allow higher concentrations of problem chemicals to be treated without inhibition. Although desirable, acclimation of methanogenic bacteria does not necessarily have to occur for the system to be effective. For example, a culture of acid-forming bacteria might be developed to partially degrade the inhibitory material to maintain a low enough concentration in a continuously fed system so that inhibition problems with methane bacteria would not result. Such a system could occur either as a completely mixed reaction system with a rapid decomposition of inhibitors or in a staged system with inhibitory

material degradation in the first stage and methane decomposition in subsequent stages.

The previously cited screening-type data on inhibition obtained using a Warburg respirometer are representative of inhibitors to methanogenic bacteria under spill or other shock-loading conditions. In order to assess the chemical's effect on the methanogenic bacterial culture when received on a regular basis tests with biomass which has had an opportunity to adapt or acclimate are necessary.

Two types of studies were conducted regarding anaerobic bacterial acclimation to chemicals inhibitory to methane production. In the first type, attempts were made to acclimate the methane organisms per se to selected inhibitory materials. In a second series of experiments, inhibitory materials were treated in continuous flow, packed-bed reactors to evaluate the ability of the total anaerobic biomass to acclimate to and degrade the material. Chemicals were selected for acclimation testing based primarily on their presence in known inhibitory waste flows in large concentrations.

Batch-Fed Anaerobic Digestion Studies

Test Series #1 Acclimation studies were initiated using the reactors shown in Figure 17. Within these reactors an anaerobic biomass was subjected to slowly increasing amounts of a specific chemical structure in the feed. An initial test series was set up to develop suitable sampling and refeeding techniques. Sampling and refeeding of the unit was accomplished 23 times without any loss of activity in the control units which received no inhibitory materials. A detailed experimental procedure is presented in Appendix I.

Test Series #2 Based on the results of this first test series several modifications were made in the subsequent test procedures. The major changes consisted of continual mixing, which replaced the twice daily mixing. Experimental apparatus is illustrated in Figure 18 while a detailed procedure is included in Appendix I.

FIGURE 17
BATCH-FED ANAEROBIC REACTORS

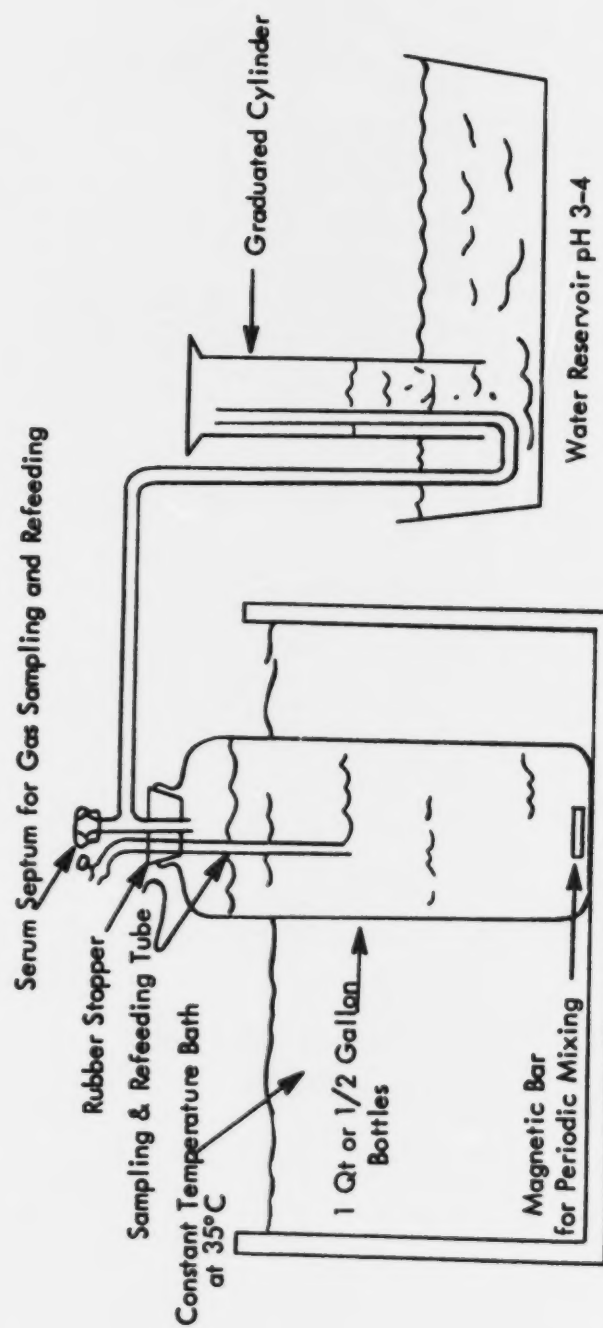
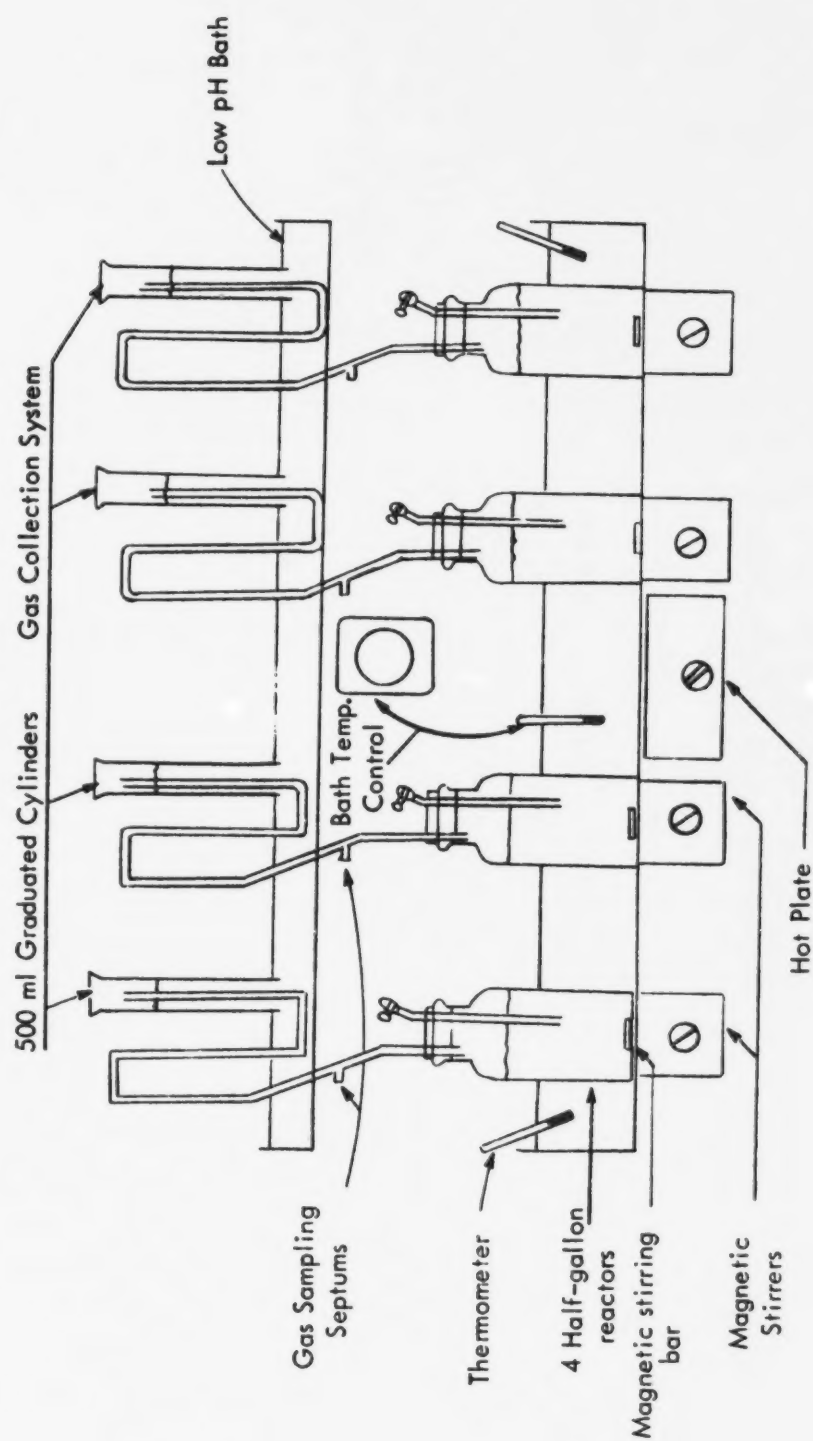


FIGURE 18

BATCH-FED

COMPLETELY MIXED ANAEROBIC REACTORS



Once the desired test chemical dosage was achieved and gas production remained suitable, biomass samples were removed from these mixed acclimators for use in evaluation tests in a Warburg respirometer to measure the degree of acclimation obtained by methanogenic bacteria. The same Warburg procedures were utilized with these acclimated sludges as were employed in the previous inhibition testing.

Acclimator Data

The gas production data obtained from the initial series of batch-fed acclimation tests are presented in Figures 19 through 21 as compared to the degradable calcium acetate control. The two test systems fed a degradable substrate remained active over an extended period through many sampling and refeeding operations. No notable difference was observed between the two control units. Although the sampling and refeeding technique was a success, the process failed to achieve the ultimate goal of acclimation with ethyl acrylate and methyl ethyl pyridine while one of the crotonaldehyde reactors did acclimate. As presented in Figure 19, one unit receiving crotonaldehyde was acclimated to a 80 mg/l (concentration when introduced to test bottle) dosage while the other unit exhibited complete inhibition of gas production at the 70 to 80 mg/l dosage. It should be noted that the applied inhibitory concentrations are those which would occur when the material is mixed with the contents of the reactor. If no degradation of the material occurs, the actual concentration in the reactor will build to some greater level which must be determined chromatographically. The unit which acclimated did undergo a lag period but rapidly recovered after cessation of the crotonaldehyde feed. No recovery was noted in the inhibited unit. Crotonaldehyde was found only once by chromatographic analyses (Table 8) within the unit samples taken before feeding - indicating no residual accumulation from feeding to feeding and a degradation within the unit. The 70 to 80 mg/l inhibitory level agrees well with the inhibitory concentration found in the non-acclimated biomass studies.

FIGURE 19
ACCLIMATION STUDIES WITH CROTONALDEHYDE
Test Series #1 - Intermittent Mixing

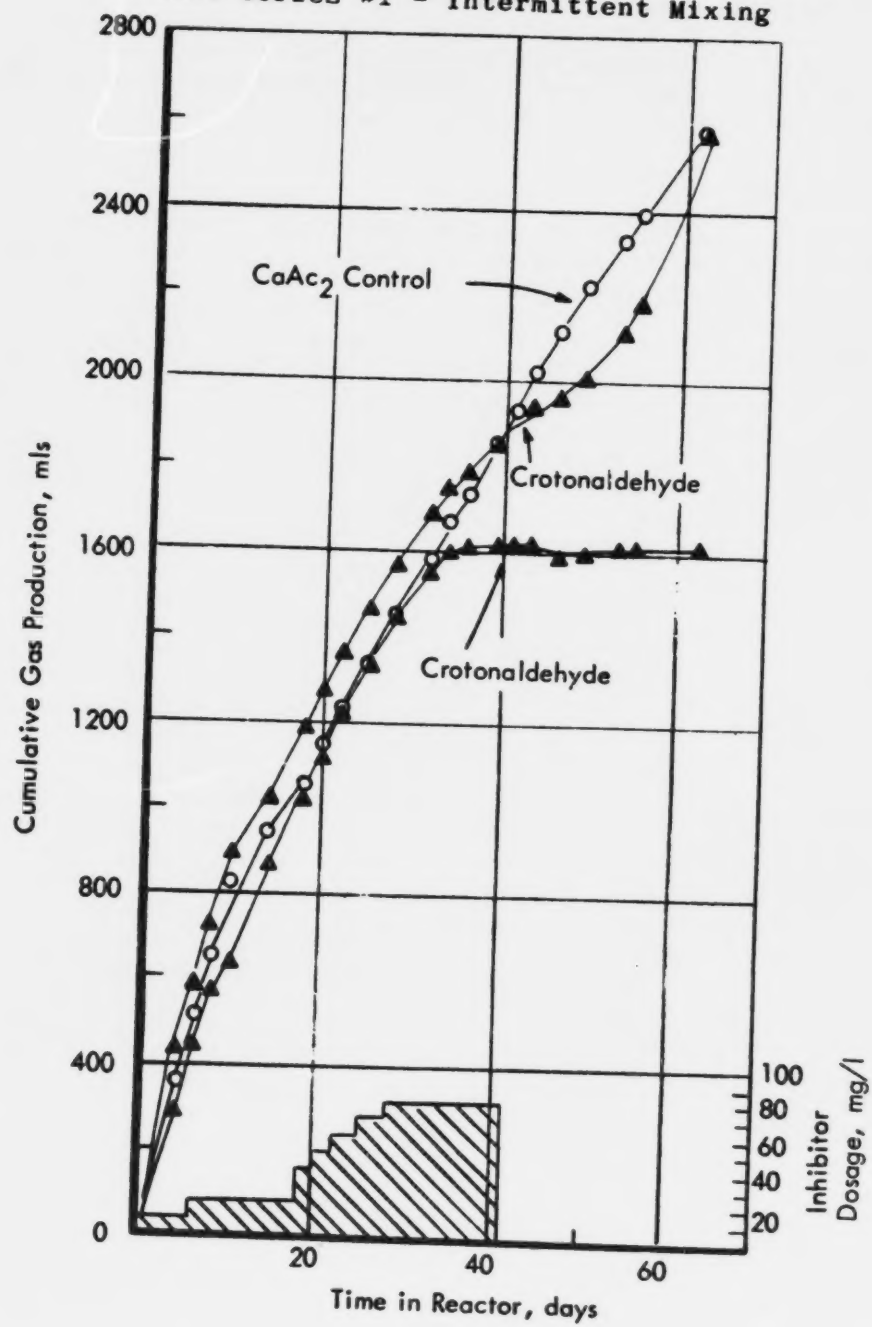


FIGURE 20

ACCLIMATION STUDIES WITH ETHYL ACRYLATE

Test Series #1 - Intermittent Mixing

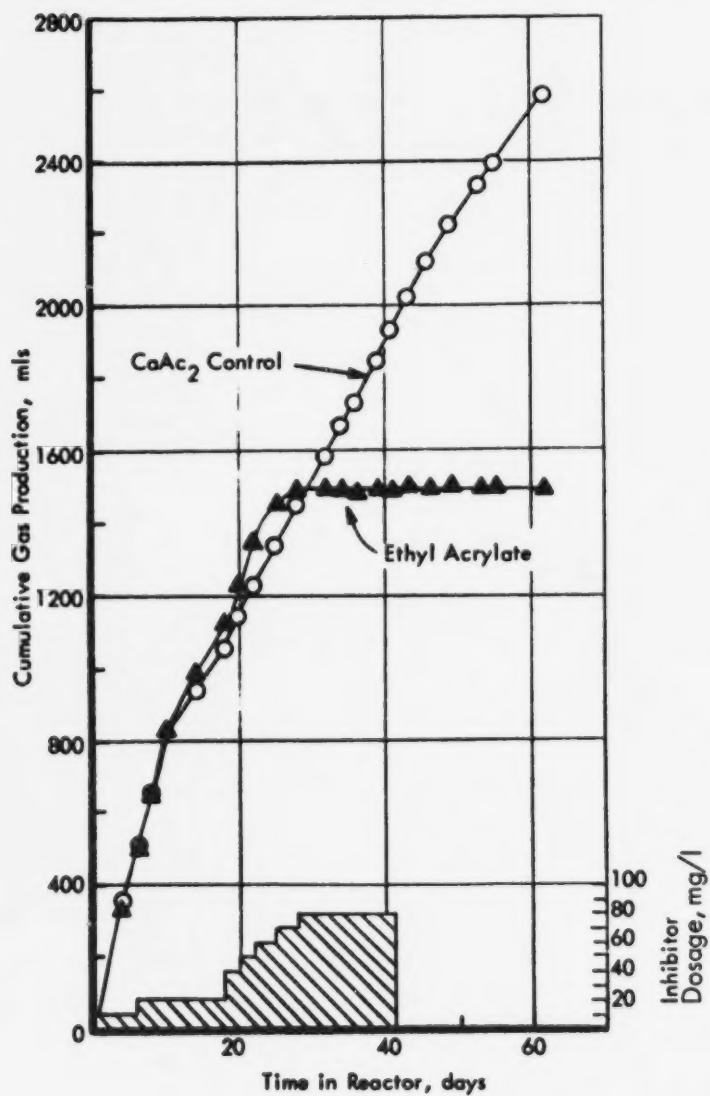


FIGURE 21
ACCLIMATION STUDIES WITH METHYL ETHYL PYRIDINE
Test Series #1 - Intermittent Mixing

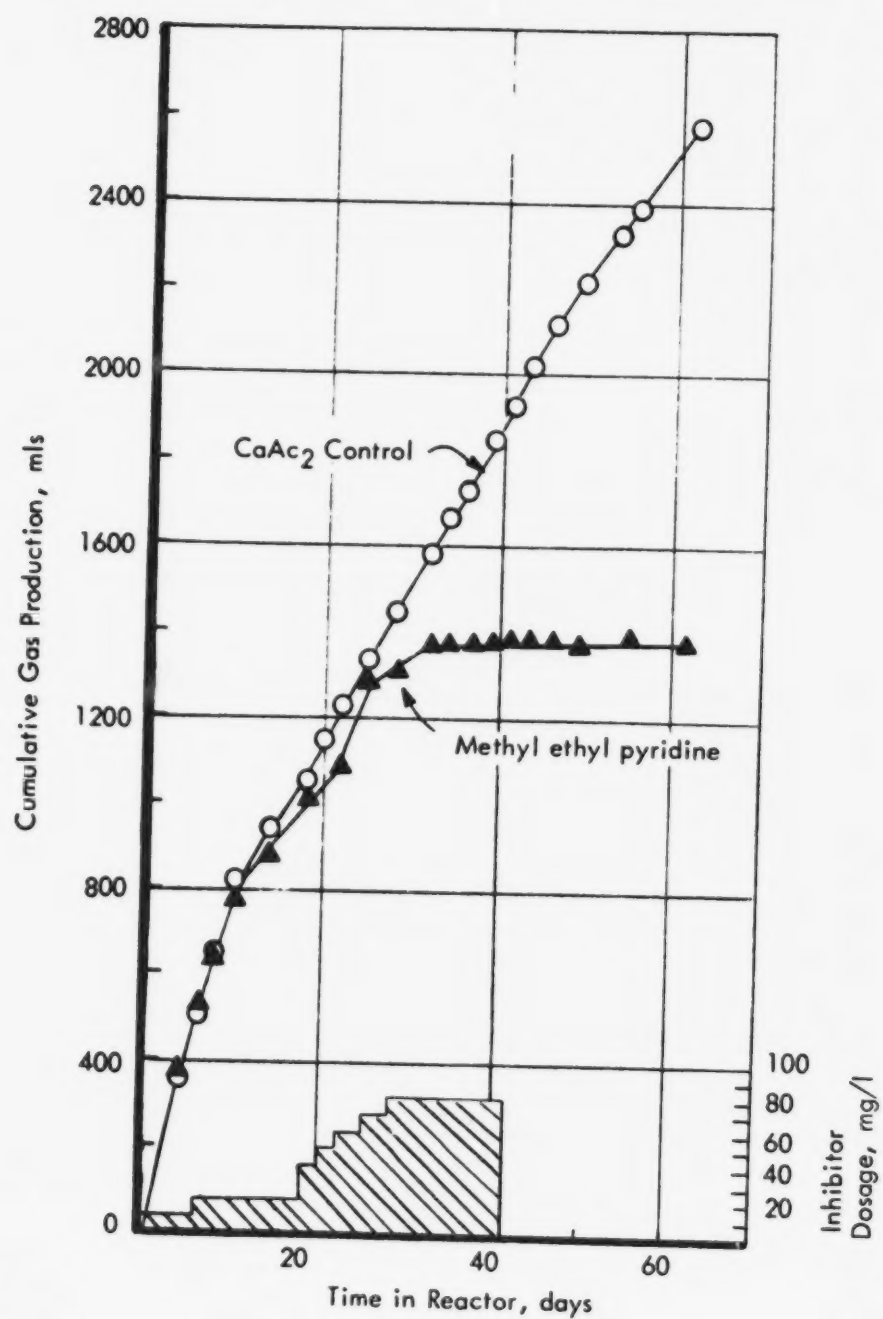


TABLE 8
SUPPORTING ANALYTICAL DATA ON BATCH-FED
ACCLIMATION REACTORS

Test Day	Units:	Chromatographic Data, mg/l		
		<u>Crotonaldehyde</u>	<u>Ethyl Acrylate</u>	<u>Methyl Ethyl Pyridine</u>
21		nil	nil	43
26		nil	nil	117
31		nil	21	312
34		nil	12	140
38		nil	20	332
45		39	nil	720
62		nil	nil	1080
69		nil	nil	960

Periodic checks of unit pH indicated it remained between 6.5 and 7.5 during the entire test.

Limited alkalinity data showed a range of between 2200 and 3600 mg/l as CaCO_3 .

Volatile acid data showed a range between 700 and 3000 mg/l as HAc during the study.

Ethyl acrylate exhibited inhibitory properties to both experimental units at reactor concentrations of 60 to 70 mg/l, while methyl ethyl pyridine showed problems at the 40 to 50 mg/l feed level. Chromatographic data on periodic acclimator samples showed only a small test-chemical buildup in the ethyl acrylate bottle indicating some degradation of this structure, as noted in those systems treating crotonaldehyde. Similar data on the bottles containing methyl ethyl pyridine (MEP) indicated a fairly rapid increase in residual concentration of the test chemical. While the apparent inhibition occurred at the 40 to 50 mg/l dosage level, an actual concentration of 117 mg/l was present in the bottle. The inhibitory concentration of ethyl acrylate found in the acclimation tests was less than that observed in non-food limited tests with unacclimated biomass (60 to 70 versus 40 mg/l) while the methyl ethyl pyridine concentration remaining in the bottle was close to the 100 mg/l concentration which proved inhibitory in screening tests.

Based on the results of this first acclimation effort, the continuously mixed acclimation system was employed in Test Series #2. The gas production data presented in Figures 22 through 25 for the continuously mixed test series indicate some acclimation of the culture to crotonaldehyde, while in the units fed phenol, sodium acrylate and ethyl acrylate, inhibitory levels were not reached. The acclimation unit receiving crotonaldehyde produced more cumulative gas than did the control due to a leak in the control during the first days of the experiment.

A Warburg respirometer test utilizing cultures from the control and crotonaldehyde units on Day 84 of the acclimation period confirmed that the culture was acclimated to crotonaldehyde dosages of at least 200 mg/l. These results are shown in Figure 26 along with previous Warburg inhibition data on crotonaldehyde. All Warburg studies were performed as previously described over a 12-hour test period.

The gas production data shown in Figures 23 through 25 still indicate inhibition with sodium acrylate (gas production lower than control) while a degree of acclimation apparently was achieved with phenol and ethyl acrylate. Warburg respirometer studies

FIGURE 22
GAS PRODUCTION IN ACCLIMATION UNIT RECEIVING CROTONALDEHYDE
Test Series #2 - Continuously Mixed Digesters

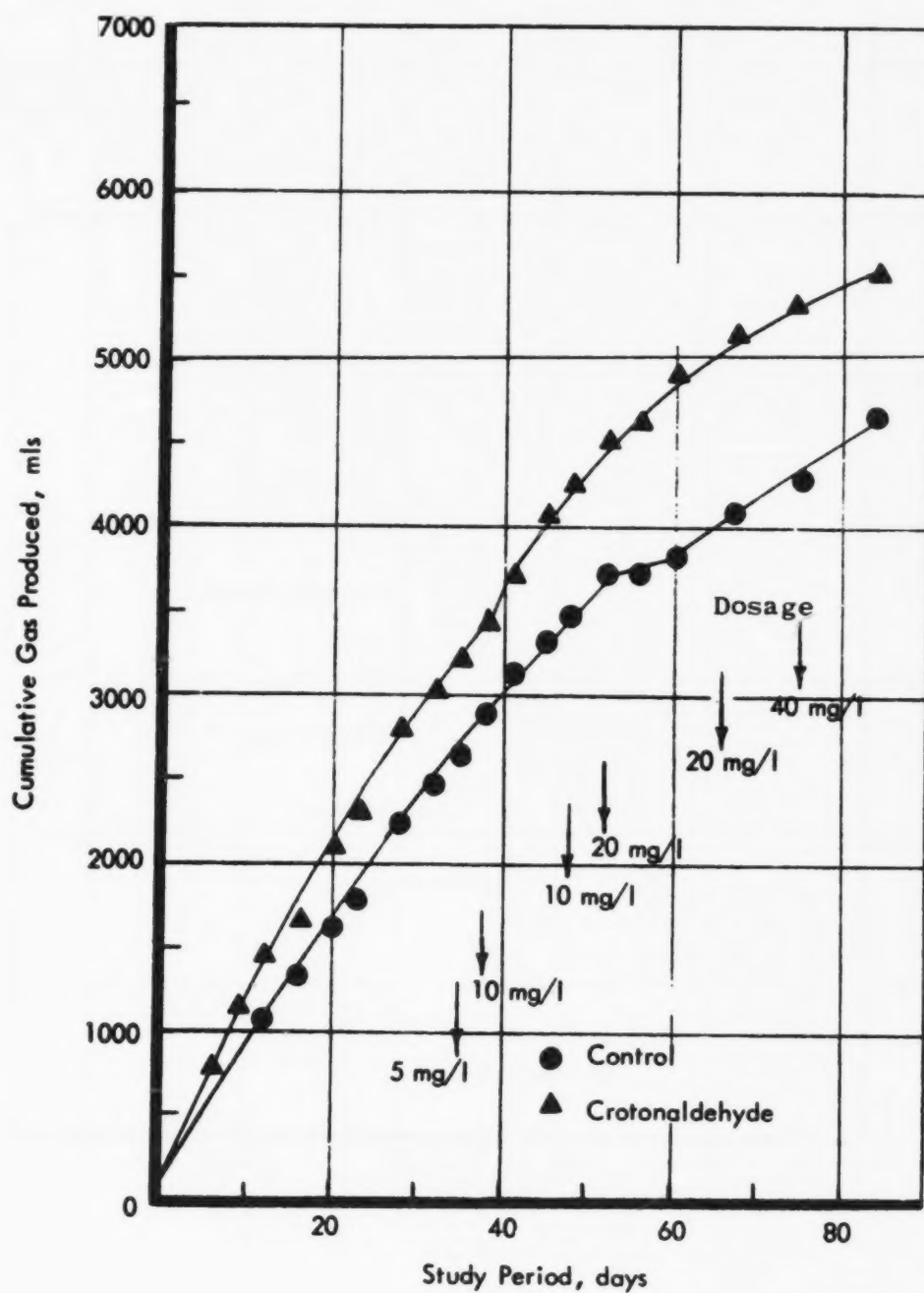


FIGURE 23
GAS PRODUCTION IN ACCLIMATION UNIT RECEIVING PHENOL
Test Series #2 - Continuously Mixed Digesters

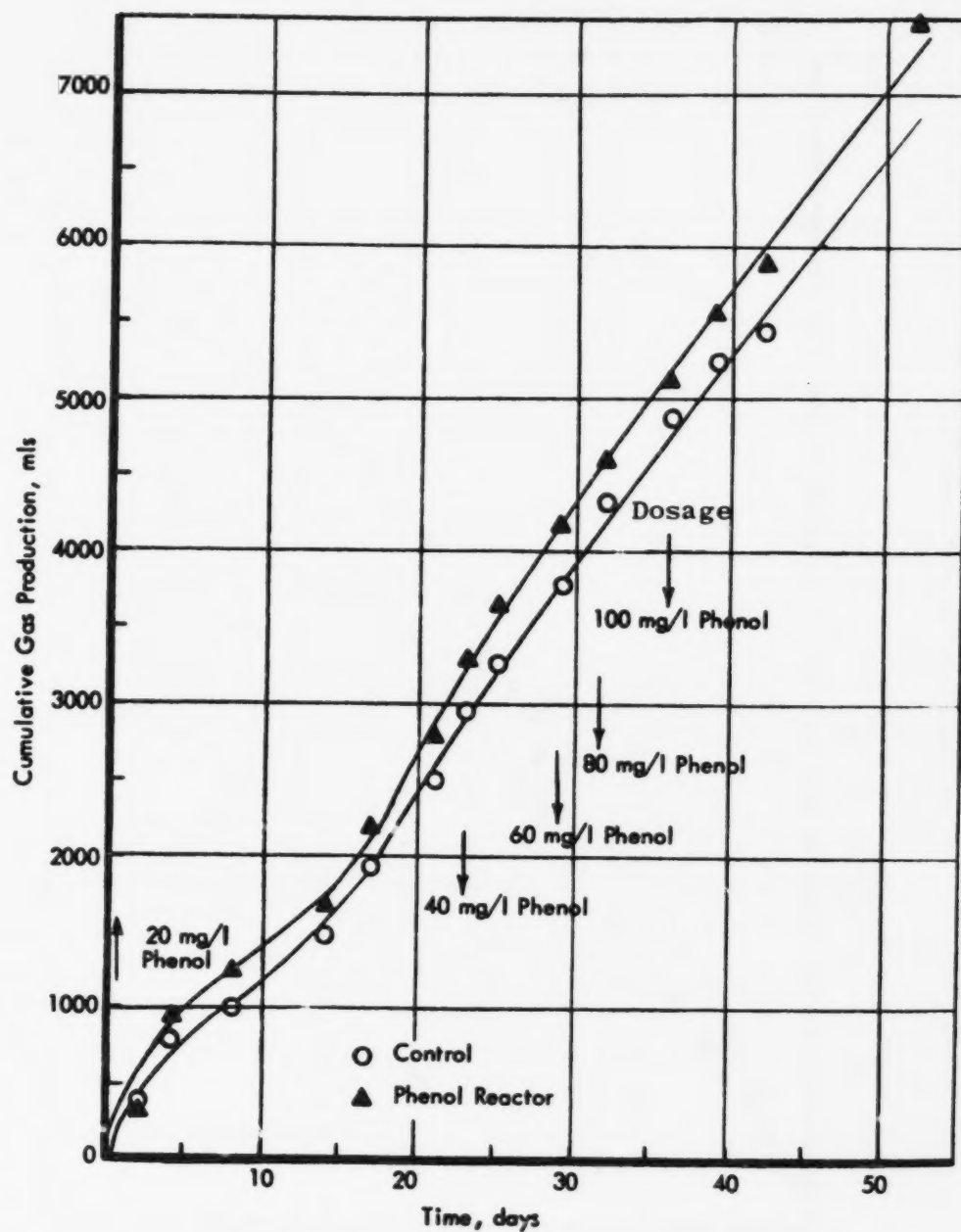


FIGURE 24
GAS PRODUCTION IN ACCLIMATION UNIT RECEIVING ETHYL ACRYLATE
Test Series #2 - Continuously Mixed Digesters

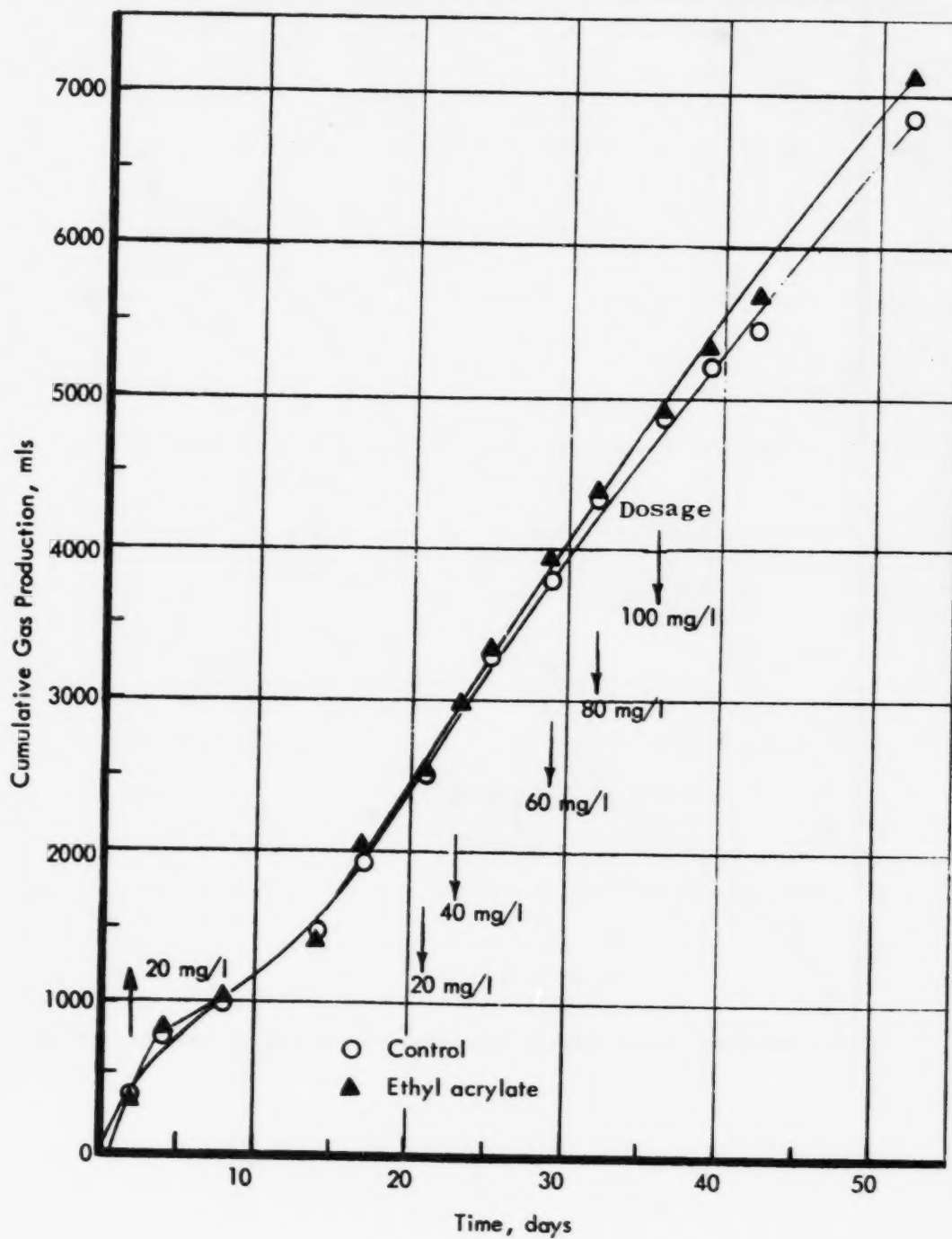


FIGURE 25

GAS PRODUCTION IN ACCLIMATION UNIT RECEIVING SODIUM ACRYLATE

Test Series #2 - Continuously Mixed Digesters

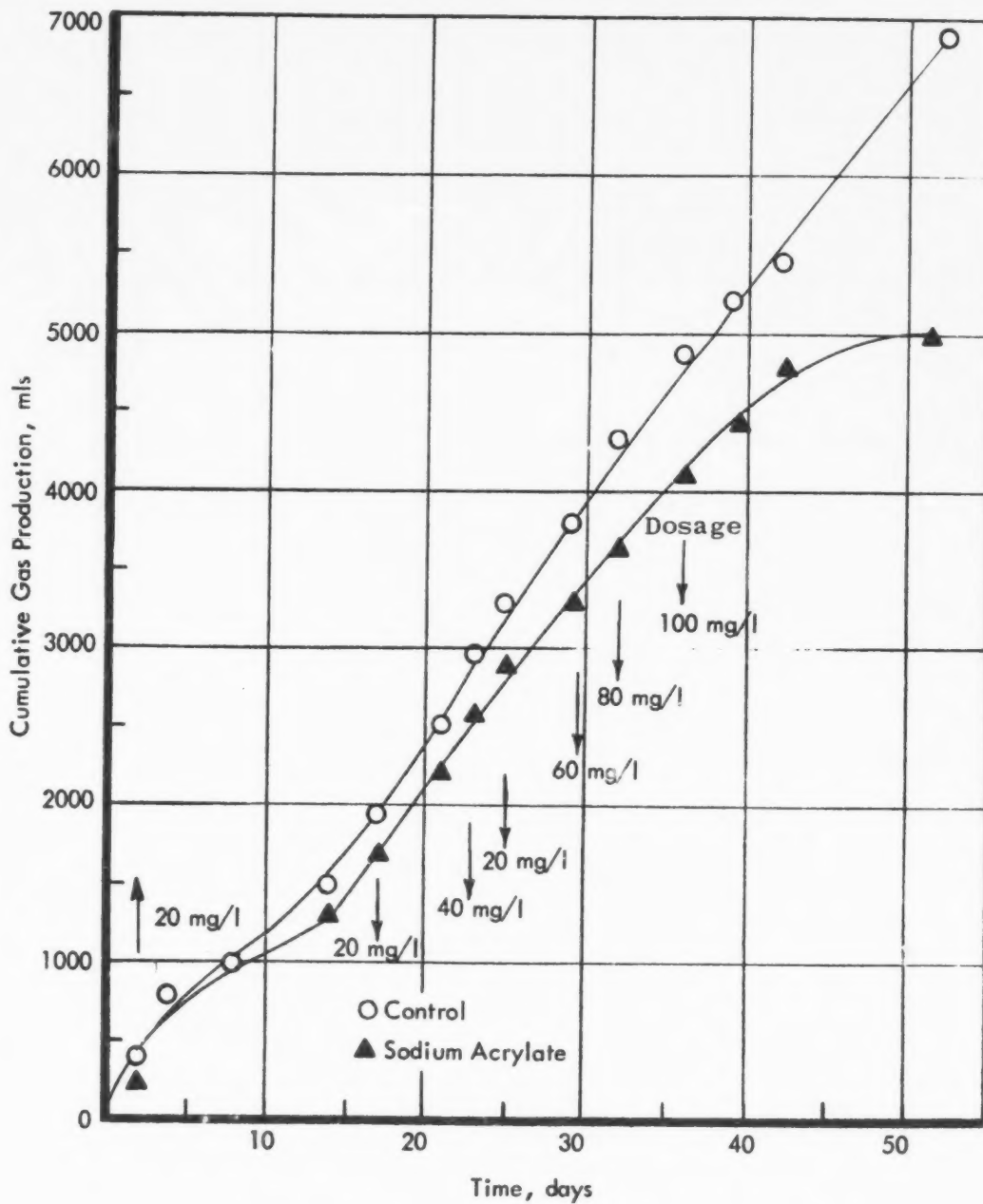
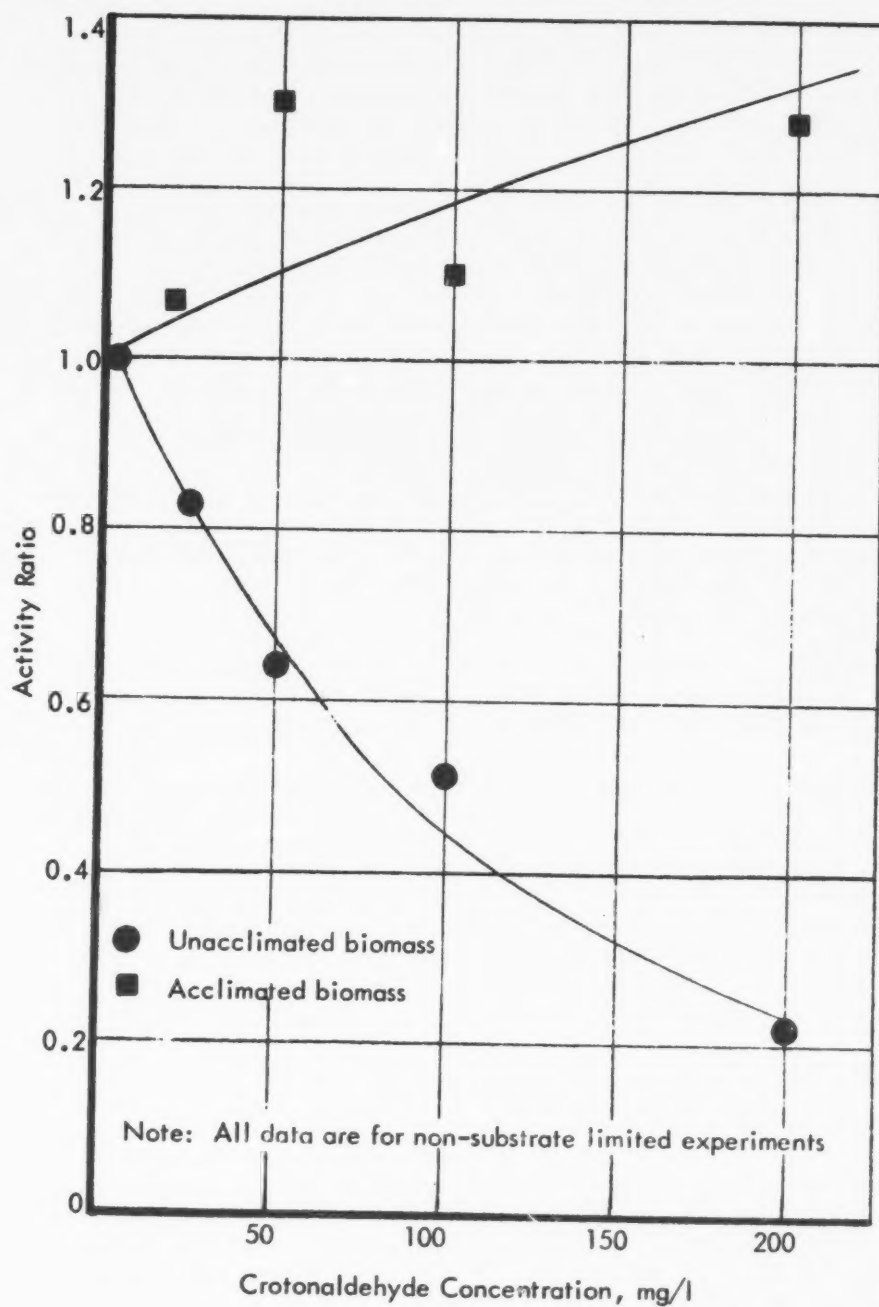


FIGURE 26

WARBURG TEST OF ACCLIMATION TO CROTONALDEHYDE



on these acclimated cultures performed on Day 54 of the study, (Figures 27 through 29) show that the culture was definitely acclimated to phenol at added levels of up to 400 mg/l. This 400 mg/l concentration was considerably above that fed to the acclimation unit. Consideration of the residual phenol in the reactor sludge (270 mg/l) indicates acclimation at concentrations of 635 mg/l since 50 percent by volume sludge was added to the Warburg flask.

The ethyl acrylate Warburg indicated a decreased inhibition at concentrations of 50 and 100 mg/l levels (the maximum concentration to the acclimation unit) but an activity similar to that in non-acclimated reference sludge and domestic sludge at 200 mg/l. Thus an acclimation was seen to the level of material steadily fed but none above this concentration. No residual ethyl acrylate was found in the reactor by chromatographic analysis indicating that the material degraded.

No increase in resistance to sodium acrylate inhibition was noted with the acclimated biomass. This might be predicted from the gas production in the acclimation reactors which was noted to be lower than that in both the calcium acetate control and the units receiving phenol and ethyl acrylate. Leakage in the highest concentration of sodium acrylate listed in the Warburg experiment may account for the greater inhibition over that seen in the non-acclimated experiments.

A data summary of the acclimation of methane bacteria to the four materials is presented in Table 9. Information on the maximum concentrations of crotonaldehyde and phenol which could be tolerated by the acclimated biomass would be desirable, however, once the acclimation reactors had been opened for sludge removal it was considered undesirable to reuse the sludge for further Warburg studies due to oxygen contamination and resulting toxicity.

Packed Column Studies

The anaerobic packed column reactor (1, 15) was chosen for testing inhibition of selected chemicals in an on-line treatment system due to the stability of operation of laboratory units of this type, the plug-flow tendency of this type reactor, and the potential economic advantage of this type anaerobic system over the complete mix, digestion unit (1).

FIGURE 27
WARBURG TEST OF ACCLIMATION TO PHENOL

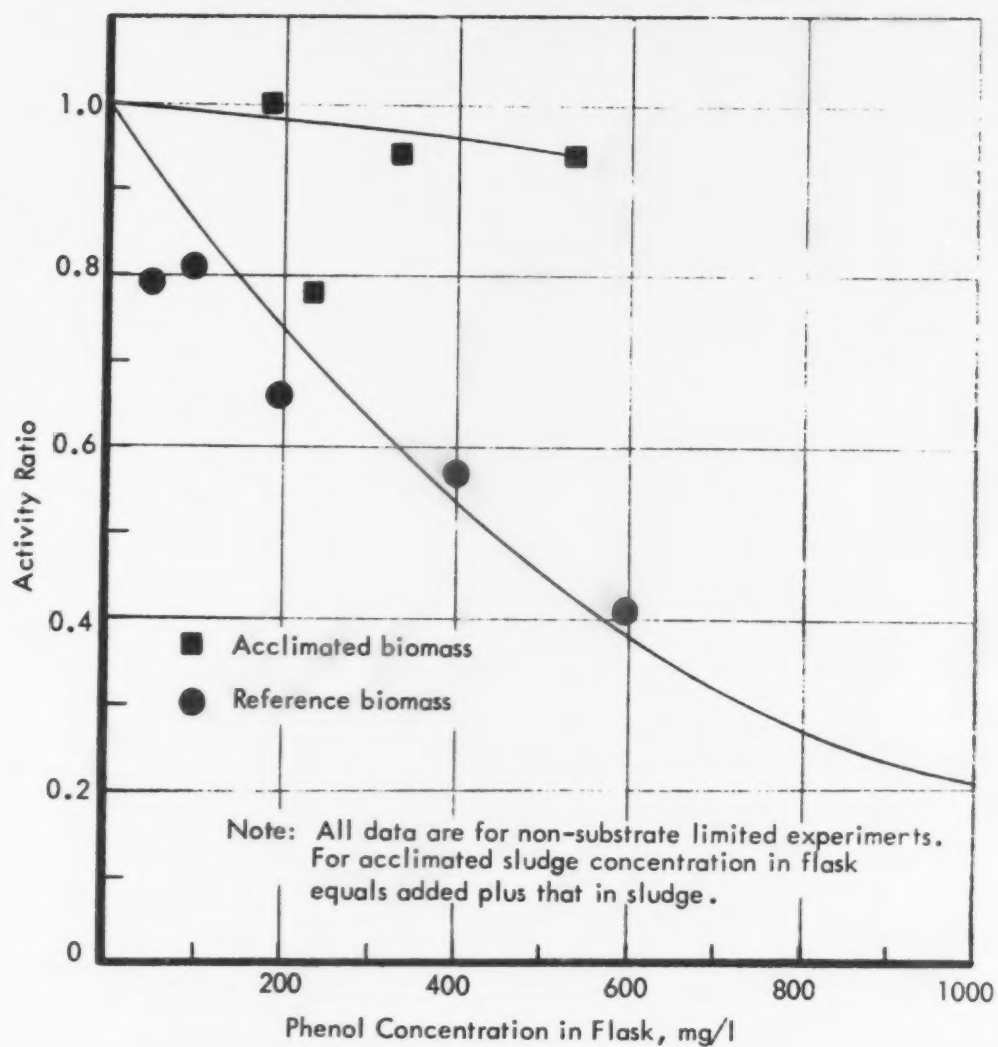


FIGURE 28
WARBURG TEST OF ACCLIMATION TO ETHYL ACRYLATE

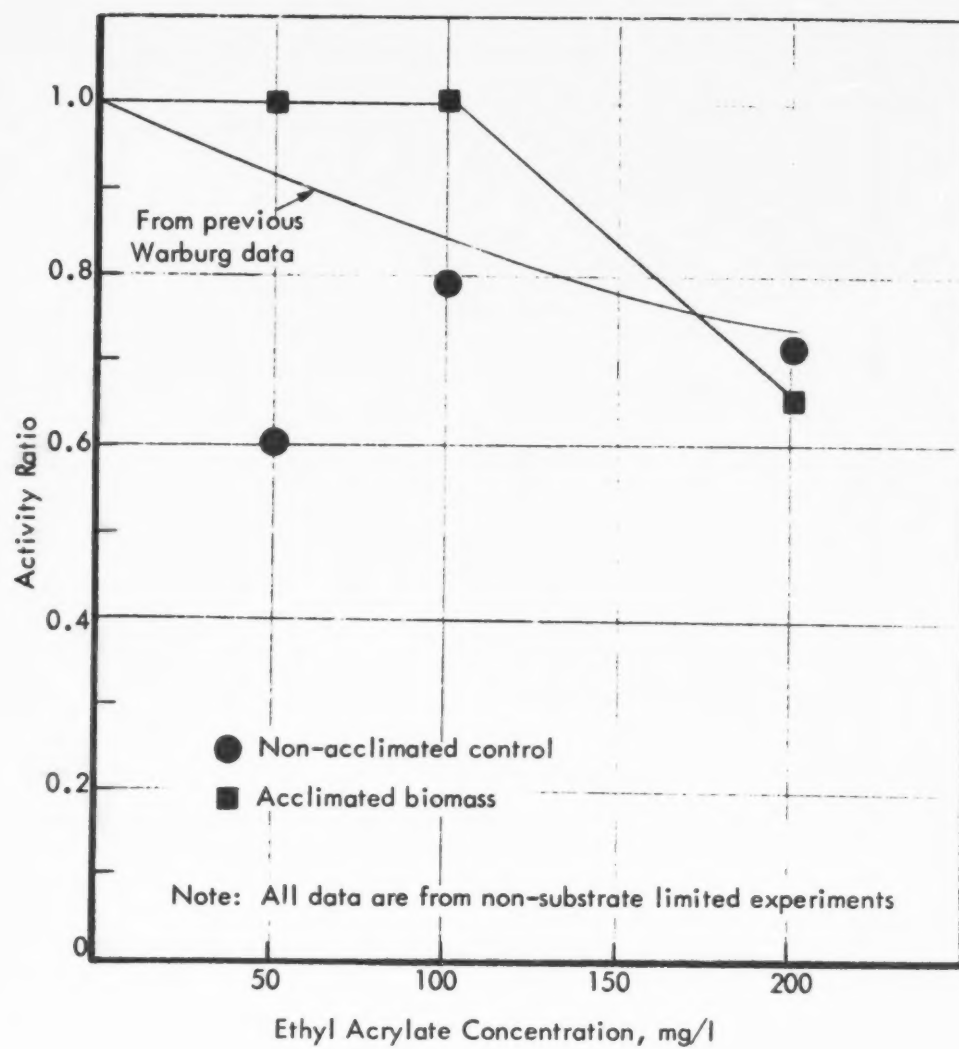


FIGURE 29

WARBURG TEST OF ACCLIMATION TO SODIUM ACRYLATE

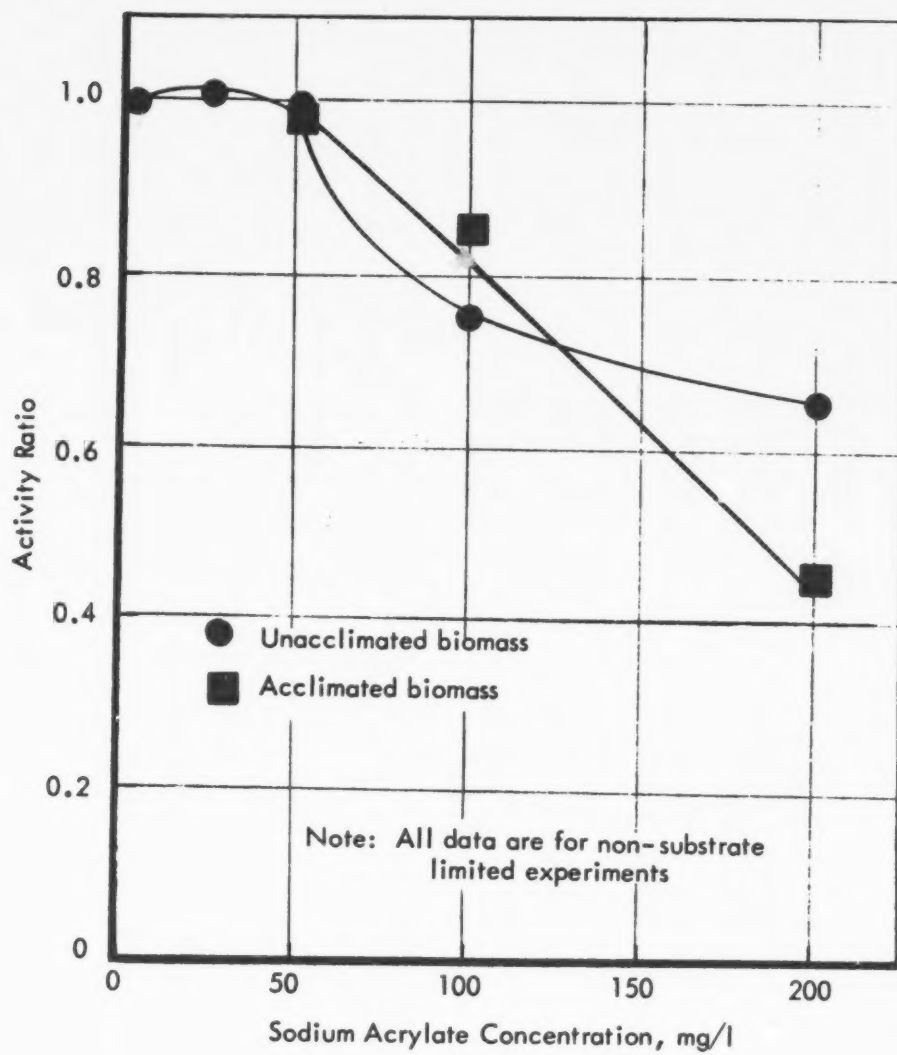


TABLE 9

WARBURG RESPIROMETER EVALUATION OF ACCLIMATION ACHIEVED FOR CROTONALDEHYDE, PHENOL, SODIUM ACRYLATE, AND ETHYL ACRYLATE IN CONTINUOUSLY MIXED REACTORS

Chemical Tested	Source of Biomass (a)	Activity Ratio at Various Test Chemical Concentrations									
		20	50	100	200	300	400	500	600	1000	
Crotonaldehyde	(1) Non-acclimated	0.85	0.65	0.31	-	0.21	-	-	-	-	-
	(2) Non-acclimated ref.	-	0.58	0.46	-	-	-	-	-	-	-
	(3) Acclimated	1.07	1.30	1.10	1.27	-	-	-	-	-	-
Phenol	(1) Non-acclimated	-	0.97	0.72	-	0.69	-	-	-	0.22	-
	(2) Non-acclimated ref.	-	0.79	0.81	0.66	-	0.52	-	0.41	-	-
	(3) Acclimated (b)	-	1.00	0.78	0.94	-	0.94	-	-	-	-
Sodium Acrylate	(1) Non-acclimated	1.02	1.03	0.74	-	-	-	0.54	-	-	-
	(2) Non-acclimated ref.	-	0.98	0.71	0.51	-	-	0.19	-	-	-
	(3) Acclimated	-	0.97	0.85	0.43(c)	-	-	-	-	-	-
Ethyl Acrylate	(1) Non-acclimated	-	-	0.77	-	0.63	-	-	0.35	0.21	-
	(2) Non-acclimated ref.	-	0.60	0.79	0.71	-	-	0.38	-	-	-
	(3) Acclimated	-	1.00(d)	1.00(d)	0.65	-	-	-	-	-	-

(a) All sludges were originally obtained from an active primary digester at the Charleston sewage treatment plant. The three sludges reported here are:

(1) Sludge used directly in the Warburg tests without any type of acclimation, i.e., the screening Warburg test series.

(2) Sludge maintained as test reference in laboratory mixed digesters with a calcium acetate and buffer/nutrient feed while companion units were being fed the test chemical. Although some reduction in activity of this sludge was noted during this extended maintenance period, the activity ratio measurements showed agreement with the previous Warburg screening tests.

(3) This sludge was maintained as (2) except a test chemical was gradually introduced to the laboratory unit to achieve an acclimated biomass.

(b) Background phenol concentration in the acclimated sludge was 270 mg/l.

(c) Flask was leaking during early part of test; oxygen contamination may have been toxic to the methanogenic bacteria.

(d) Since the reference flask failed to produce reasonable gas levels, the 50 mg/l ethyl acrylate test flask was utilized for calculating activity ratio.

Packed Column - Procedures

Packed-bed reactor studies were carried out with two types of equipment. The first system was a single-walled plexiglass unit warmed by heating tape, while a later system employed a larger, jacketed reactor which was warmed by water circulated from a constant-temperature bath.

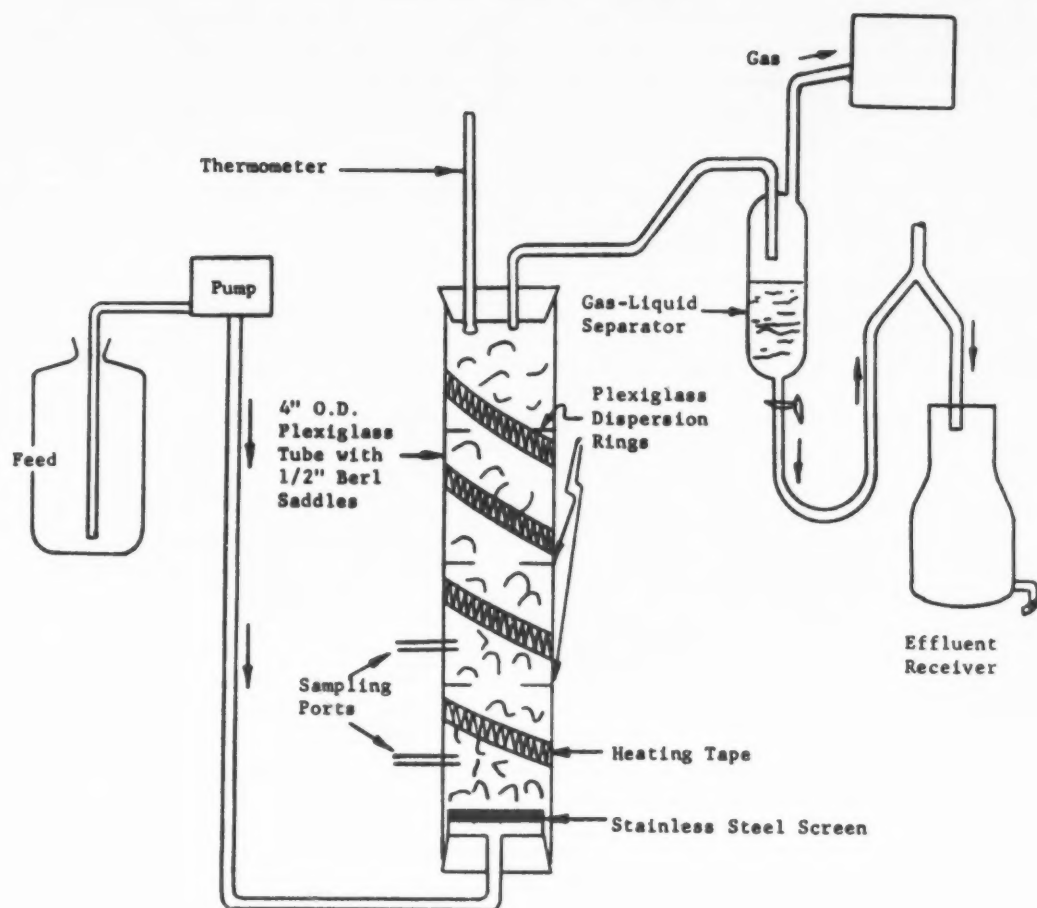
The initial design shown in Figure 30 consisted of a packed column of 3-liter void capacity. Two units of this type were seeded, with 300 ml of screened, primary digested sludge (7.7 percent total solids) from a domestic sewage treatment plant and operated on a synthetic volatile-acid feed (Feed A, Table 13) with a detention time of 1.5 days at 35°C temperature.

After startup (approximately two months) the reactors were converted to a degradable, mixed organic feed (Feed B, Table 13). Feed B was used in most of the studies with packed-bed reactors as either the initial feed or as a supplementary food supply even though later Warburg inhibition-tests show methyl isobutyl ketone to be inhibitory to non-acclimated sludge. This component may have been responsible for the seemingly excessive 3-month time interval required to achieve high reactor performance (90-95 percent COD reduction).

Operational data taken during packed-bed studies consisted of the following:

1. Chemical oxygen demand or total carbon on samples of unit feed and effluent. These samples were filtered through 0.45 μ membrane-filter paper and refrigerated until analyzed.
2. Daily gas production monitored by in-line gas meters and frequent mass spectrographic analyses.
3. An occasional gas chromatographic comparison made on the unit feed and effluent samples.
4. Reactor environmental parameters of pH, volatile acids, and alkalinity.

FIGURE 30
PACKED-BED ANAEROBIC REACTOR



In order to evaluate the actual detention time and flow characteristics in the packed-columns, dye tests were performed on a heated column free of biological solids using Rhodamine B as a tracer. The results of these tests shown in Figure 31 indicate considerable short circuiting with an actual effective detention time of 12 hours rather than the theoretical 36 hours. Visual observations during the test indicated a dye interface moving as a slug along thermal gradients created by the external heating tapes, which allowed the dye to reach a higher concentration in the effluent than in an internal sampling port.

An attempted dye study on a solids-containing packed-bed reactor failed due to excessive absorption of the dye. Biological solids in the unit might reduce some of the short circuiting, but should not change the general trend of these results. The dye studies indicate that although short circuiting exists, the feed was not mixed through the column but retained partial identity as a slug, at least during the first twelve hours of the test. Volatile acid and specific organic profiles obtained through the column (Table 10) are further indicators that complete mixing did not exist in the operating columns.

To approximate more nearly a plug-flow system, a double-walled or jacketed plexiglass reactor was designed to eliminate the temperature gradients observed in the single-walled reactor equipped with heating tape. Water from a constant-temperature bath was circulated through the jacket to maintain an even temperature.

Studies using the anaerobic packed columns progressed in the following fashion. During the first phase one column was used as a control treating the degradable Substrate B in Table 13 to achieve a performance base while the other received this feed plus a slowly increasing dosage of crotonaldehyde. The objective was to compare the acclimation in an on-line, bench-scale treatment system to that seen in the previously cited methanogenic batch fed studies performed in continuously-mixed digesters.

FIGURE 31
DYE STUDY ON PACKED-BED REACTOR

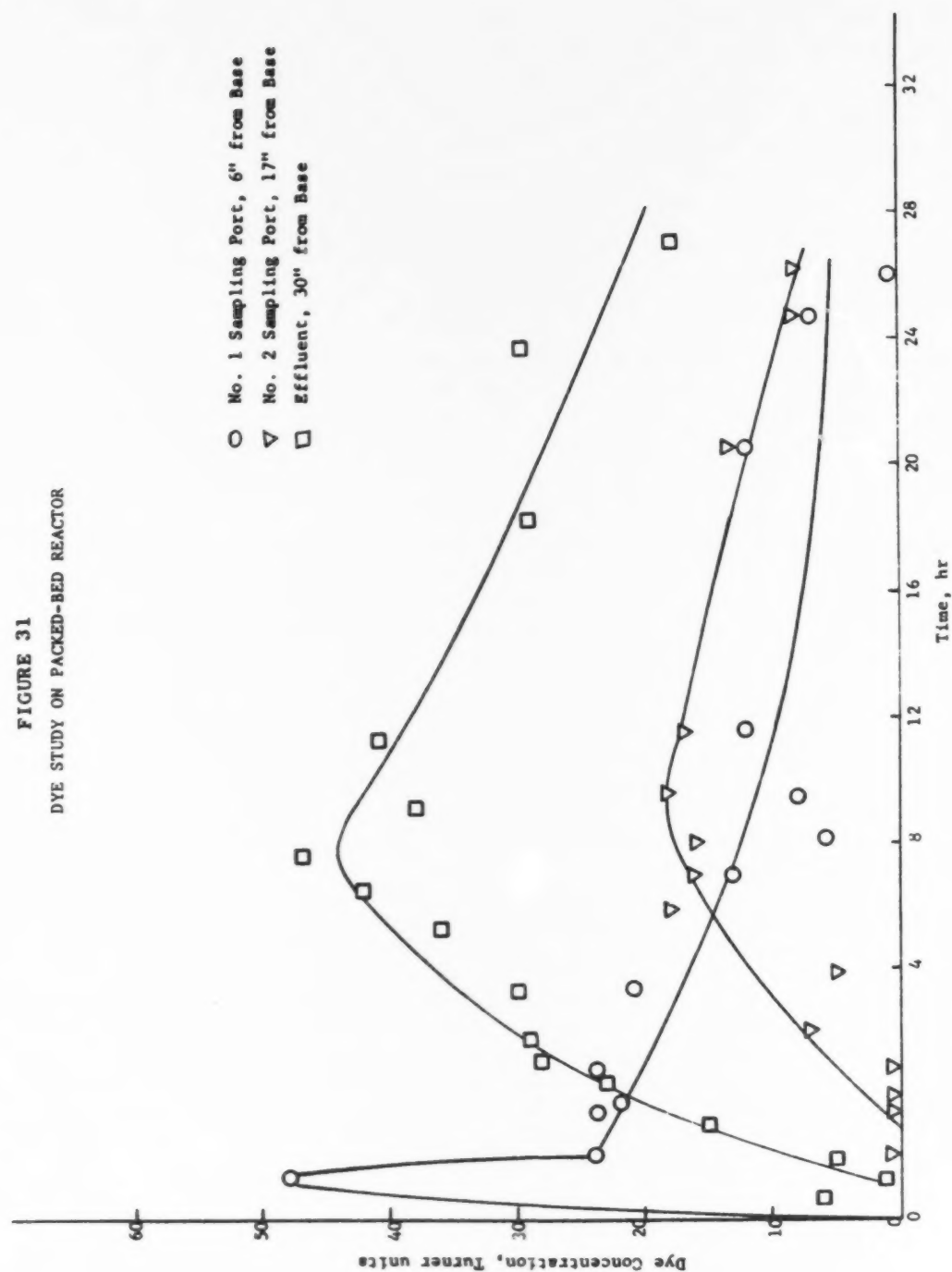


TABLE 10
VOLATILE ACID AND SPECIFIC ORGANIC PROFILES
IN OPERATING PACKED COLUMNS

	Methyl Isobutyl Ketone, mg/l	Crotonaldehyde, mg/l	Volatile Acids, mg/l as HAC
Feed ^(a)	132	525	570
6" from inlet	19	nil	325
15" from inlet	5	nil	nil
Effluent ^(c)	nil	nil	nil
Feed ^(b)	--	--	600
6" from inlet	--	--	176
15" from inlet	--	--	nil
Effluent	--	--	nil

(a) Unit receiving mixed degradable feed plus crotonaldehyde

(b) Unit receiving volatile acid feed

(c) Both columns are 30 inches total height

During the second study, a combination of four inhibitors (formaldehyde, ethyl acrylate, phenol, and acrylonitrile) was slowly introduced to a column to the point of complete inhibition (as measured by reduction COD removal and gas production); the effluent from this column was fed to another column for possible further treatment.

The final phase utilized the performance of the control observed in the first studies as compared to the performance in treatment of two process unit waste streams from one of Union Carbide Corporation's petrochemical manufacturing facilities. These wastes were introduced over a period of time to allow acclimation to inhibitory materials known to be present.

Packed-Bed Results

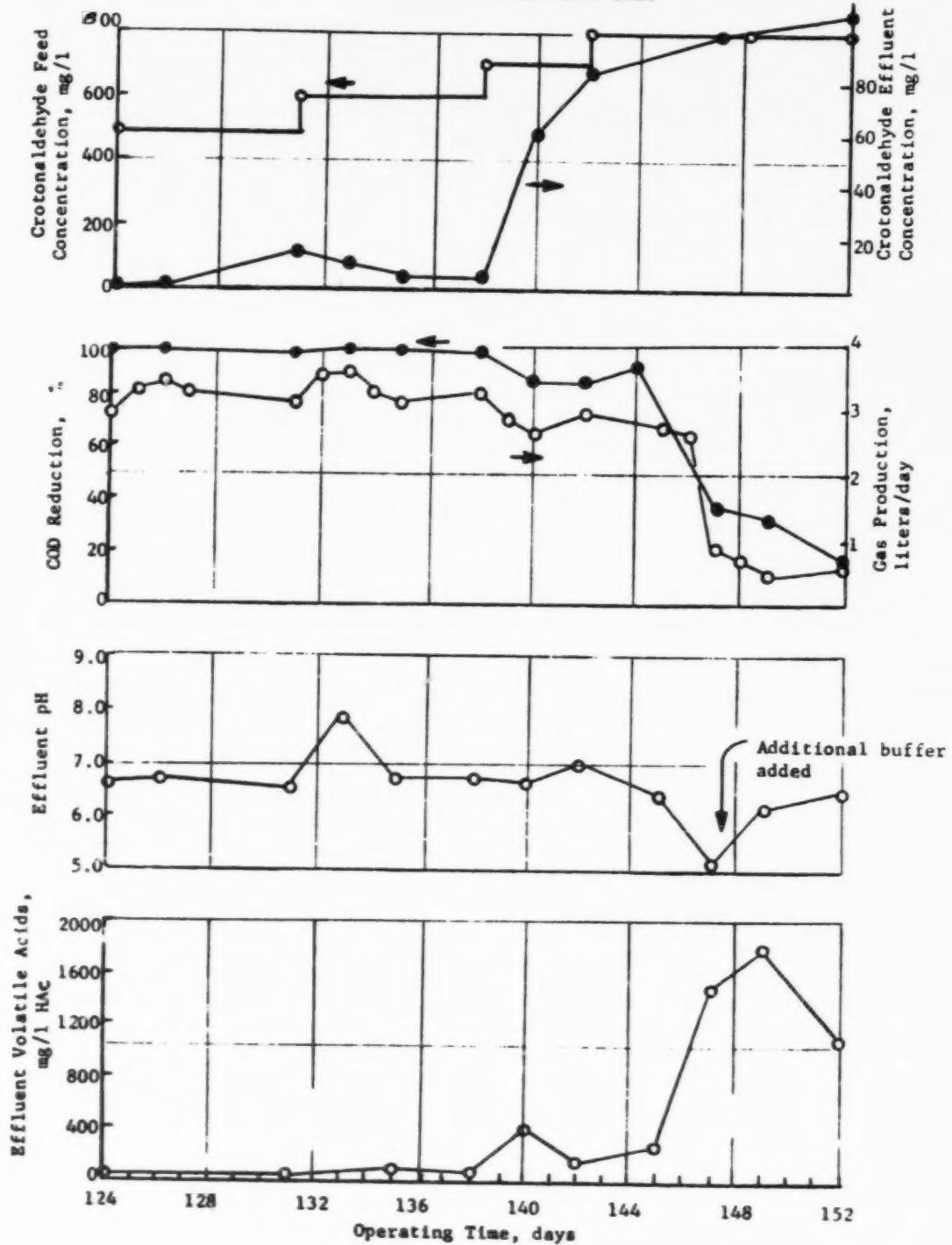
The control unit receiving the Synthetic Feed "B" provided a baseline removal of 90 to 98 percent of the applied COD while operating at a temperature of 35°C, a detention time of 1.5 days, and a volumetric loading of 110 to 130 lb COD/1000 ft³-day. Gas production for this unit ranged from 1.5 to 2 liters/day.

Crotonaldehyde Unit - Performance of the packed column receiving the synthetic plus crotonaldehyde both under steady operating conditions and during failure are illustrated in Figure 32. The unit had successfully received up to 500 mg/l of crotonaldehyde in gradually increasing (over 132 days) dosage with no adverse effects. During this time the COD reduction was comparable to that observed in the control unit and gas production was in excess of that in the control, both indicating degradation of the crotonaldehyde.

Preliminary operational problems developed on Day 140 after two days of 700 mg/l crotonaldehyde feed. A drop in both gas production and COD removal was noted at this time, and subsequent chromatographic analysis indicated considerable leakage of crotonaldehyde in the effluent. After the influent concentration was increased to 800 mg/l a complete failure of the unit was observed. The effluent pH remained within

FIGURE 32

EFFECT OF CROTONALDEHYDE ON PERFORMANCE OF PACKED-BED
ANAEROBIC TREATMENT UNIT



the desired range until about five days after the first problem indication, at which time additional buffer was required. The five-day lag indicates that the pH level was not a contributing factor in the unit upset.

The crotonaldehyde concentrations treated in this study are considerably greater than those at which inhibition was noted in both the substrate limiting (200 mg/l) and non-substrate limiting (150 to 200 mg/l) unacclimated Warburg experiments. Although the upper limit of an acclimated system in Warburg experiments was not determined, the 600 mg/l concentration which was effectively treated was considerably greater than that tested in the acclimated equipment and should yield an effective upper limit for an acclimated system.

Effect of Combined Inhibitors

An examination of the effects of combined inhibitors was made in a packed column in which four known inhibitors (formaldehyde, ethyl acrylate, phenol and acrylonitrile) were introduced in slowly increasing quantities (equal concentration of each) spiked into the degradable synthetic organic substrate. In the latter part of the study after the column was inhibited severely in gas production and COD removal but was still producing volatile acids, the effluent was treated in another column to determine the efficiency of the packed column as a pretreatment, detoxifying device.

Data obtained during the period of increasing concentration of inhibitory materials and subsequent failure of the column are presented in Figure 33 while data on both the first and second of the series units are presented in Table 11. System performance was inhibited initially at 7.5 mg/l dosage of each material but subsequently recovered. Increasing the concentration to 25 mg/l of each material resulted in a complete cessation of gas production and essentially no COD removal. The acid-forming segment of the population was still active as evidenced by the buildup in effluent volatile-acid concentration. During the period of time that a 25 mg/l inhibitor concentration or less was applied to the first of the series units, the second unit was able to perform well on the effluent yielding a 70 percent COD removal and above

FIGURE 33
EFFECT OF FOUR INHIBITORS ON PERFORMANCE OF
PACKED-BED ANAEROBIC TREATMENT UNIT

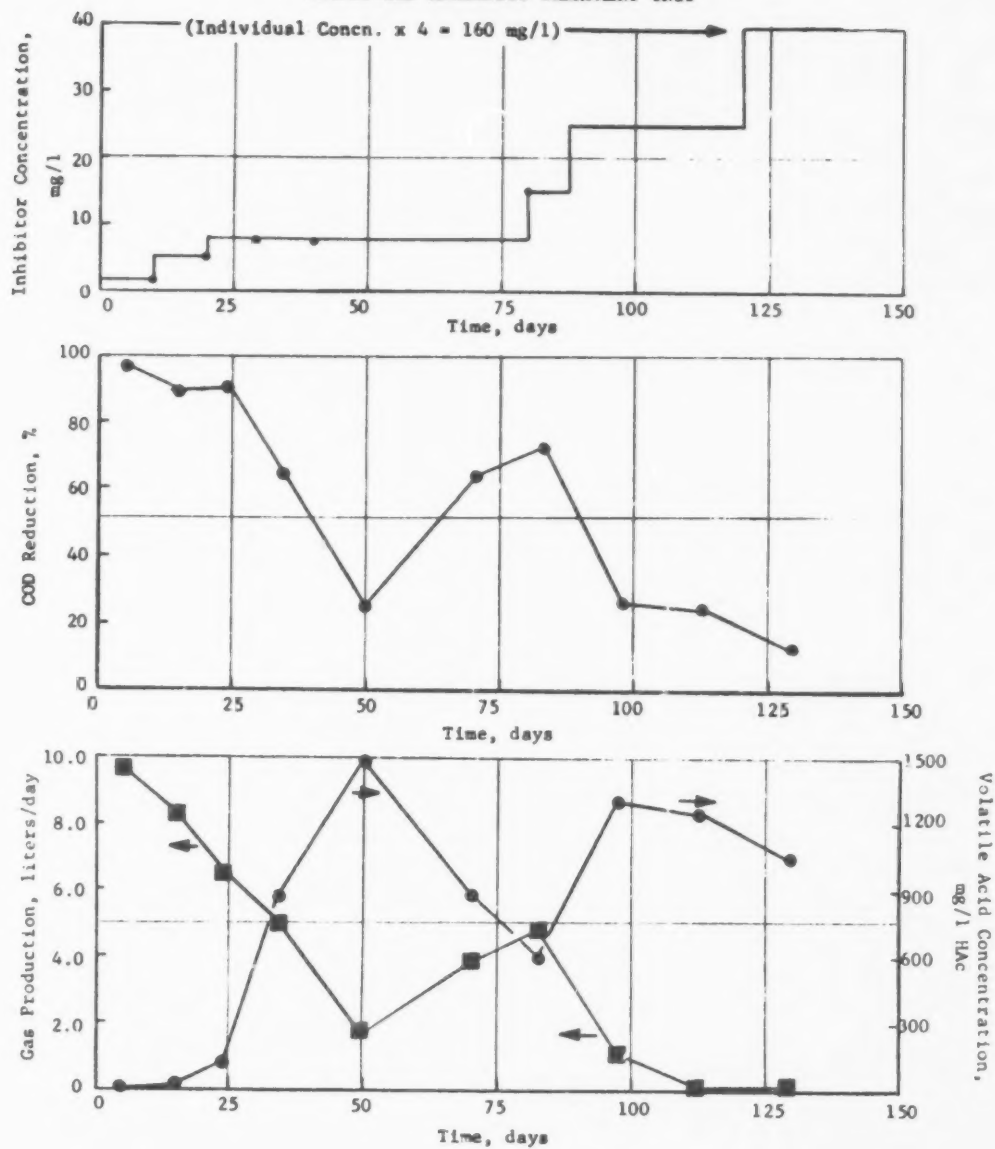


TABLE 11

PERFORMANCE OF TWO-STAGE FILTER
TREATING COMBINED INHIBITORY CHEMICALS

Period of Study and Remarks	Unit Loading, lb COD/day/ sq ft	Total Gas Production, l/day				pH				COD, mg/l				Reactor Effluent				Gravimetric Reductions, % (COD Basis)			
		Unit 1		Unit 2		Unit 1		Unit 2		Unit 1		Unit 2		Unit 1		Unit 2		Unit 1		Unit 2	
		Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2	Unit 1	Unit 2
0-10 Unit 1 treating a synthetic feed containing a gradually increasing amount of four combined inhibitors (100 mg/l each) (100 mg/l each)	205	-	9.71	-	7.0	-	-	-	-	4870	-	7.6	-	114	-	96	-	-	-	-	-
10-20 Unit 1 treating a synthetic feed containing a gradually increasing amount of four combined inhibitors (100 mg/l each) (100 mg/l each)	205	-	8.33	-	7.0	-	-	-	-	5040	-	7.6	-	505	-	90	-	-	-	-	-
20-25 Inhibitor dosage at 5 to 10 mg/l	205	-	6.48	-	7.0	-	-	-	-	4500	-	7.6	-	430	-	91	-	-	-	-	-
25-30 Continued dosage of inhibitors at 5 to 10 mg/l of each inhibitor	215	-	4.97	-	7.0	-	-	-	-	5186	-	7.3	-	877	-	65	-	-	-	-	-
30-35 Increased inhibitor dosage to 10 to 20 mg/l of each inhibitor	220	-	1.73	-	7.1	-	-	-	-	5470	-	6.2	-	1491	-	25	-	-	-	-	-
35-40 Increased inhibitor dosage to 10 to 20 mg/l of each inhibitor	216	46	3.85	1.14	7.0	7.2	1230	786	5200	1110	7.3	7.9	891	34	1806	86	65	92	92	92	92
40-45 Increased inhibitor dosage to 10 to 20 mg/l of each inhibitor	226	66	4.67	0.85	7.1	7.7	-	598	5450	1590	8.1	8.0	598	84	1462	289	73	82	82	82	82
45-50 Inhibitor dosage 20-40 mg/l of each inhibitor	225	146	1.10	1.47	7.0	6.7	-	1302	3400	3512	5.6	7.4	1302	452	3939	1035	27	70	70	70	70
50-55 Inhibitor dosage 40 mg/l of each inhibitor	226	162	0.09	2.30	7.0	5.9	-	1245	5440	4100	5.5	6.2	1245	272	4100	1200	25	70	70	70	70
55-60 Inhibitor dosage 40 mg/l of each inhibitor	225	186	0.07	1.22	7.0	6.3	-	1060	5440	4730	5.8	6.0	1060	593	4730	3011	15	37	37	37	37

in the second reactor and an 88 percent removal in both reactors. As the concentration of each inhibitor was raised to 40 mg/l the second column was inhibited. The acid-forming culture in the primary column was also inhibited as evidenced by the decrease in effluent volatile-acid concentration. An active volatile-acid-forming culture would be necessary to alter the toxic materials and prevent leakage of inhibitory materials into the effluent.

Examination of the inhibitory concentration of each of the four materials (Table 7) indicates that at the 40 mg/l feed concentration formaldehyde was near the inhibitory dosage while the ethyl acrylate, acrylonitrile, and phenol was well below inhibitory levels for the individual material. The severe nature of the inhibition observed at this concentration indicates that toxicity may be due to a cumulative effect of the four materials.

Data collected from chromatographic examination of the effluent of the two series columns on Day 125 are presented in Table 12. During this time period the second series column failed. The first column shows virtually no alteration of the applied phenol or formaldehyde, which allowed these materials to pass through and inhibit the second unit. In summary, the series operational system was found to be an effective treatment scheme for the mixed inhibitors at low concentrations, but failed with the penetration of inhibitors at higher dosage levels. The mixed inhibitors were indicated to act synergistically since they were more detrimental to unit performance as a mixture than would have been predicted based on individual chemical inhibition tests.

In order to apply the results of this study, two actual waste streams from one of Union Carbide's synthetic organic chemical plants were treated in anaerobic packed-bed reactors. These streams contained materials which were identified as inhibitory in screening studies or were detrimental to aerobic treatment. The two streams used in testing were selected so that neither associated cations nor contained sulfates would cause toxicity problems, which might complicate interpretation of any inhibition due to organic constituents. In addition, wastes selected

TABLE 12
CHROMATOGRAPHIC EXAMINATION OF SERIES PACKED COLUMNS
TREATING MIXED INHIBITORS

	Compound Concentration, mg/l			
	<u>Formaldehyde</u>	<u>Acrylonitrile</u>	<u>Ethyl Acrylate</u>	<u>Phenol</u>
Feed to first series column	40	40	40	40
First-column effluent	40	24	12	37
Second-column effluent	0	17	0	12

Gas chromatographic analysis employed the following conditions:

Column 6' x 1/8" Porapak P
Column temp.: 80°C for 2.5 min., then programmed at
 5°/min to 150°C and 10°/min to 225°C.
Carrier flow: 50 cc/min
Injection port
temp.: 250°C
Detector: Flame ionization
Detector temp: 250°C

were of reasonably low flow and high carbon concentration which would make separate anaerobic treatment a more practical consideration.

The actual waste streams selected for study are described in Table 13. The streams were modified as described below prior to feeding to the packed-bed reactors. Waste C was diluted with eight volumes of tap water to 1) provide an equivalent COD loading to previous studies, 2) to dilute known or suspected inhibitors (crotonaldehyde and acetaldehyde in particular), and 3) to yield a reasonable volatile acid level in the feed. Unit D waste was increased in strength by adding 50 percent by volume of the following mixture:

- Acrylic acid - 270 mg/l
- Acetic acid - 2430
- Acetone - 405
- Acetaldehyde - 135
- Isobutanol - 270
- Isopropanol - 270
- TERGITOL Surfactant NPX - dosage increased during course of experiment

The additives were used to compensate for dilute waste content in samples collected from the operating unit during the studies and to provide a reasonable COD loading.

Experiments treating actual waste streams were conducted in a manner similar to those with crotonaldehyde as previously cited. The reactor was first seeded with domestic anaerobic sludge and the degradable supplemental feed used to achieve a suitable culture capable of 90 to 98 percent COD reduction. The waste in question was then introduced slowly until it reached 100 percent of the reactor feed.

Unit C - Data obtained while treating the inhibitory waste are summarized in Figure 34 and Table 14. Introduction of the wastewater resulted in a reduction of performance from the 90 to 98 percent obtained with degradable Waste B to an equilibrium level of 68 percent when treating 100 percent of the diluted unit feed. A period of inhibition was initially noted at 60 percent feed strength, but continued feeding at this level resulted in a resumption of satisfactory performance. Chromatographic examina-

TABLE 13

WASTE STREAMS STUDIED IN ANAEROBIC PACKED COLUMNS

Waste A - Synthetic Volatile Acid Feed Used for Startup

COD - 3000 mg/l

990 mg/l propionic acid (1500 mg/l as COD)

1400 mg/l acetic acid (1500 mg/l as COD)

pH - 6.2 with sodium hydroxide

Tap water

COD/N/P ratio 100/1/0.2

Waste B - Degradable Mixed Organic Feed

COD - 2800 mg/l

Components

Acetic acid, ethylene glycol, ethanol,
methyl isobutyl ketone, sodium benzoate.
Added on equal COD contribution/compound

pH - 7.0 with sodium hydroxide

Tap water

COD/N/P ratio 100/1/0.2

TABLE 13 (CONTINUED)

Waste C - Inhibitory Waste

Total Carbon - 10,000 mg/l

COD - 25,000 mg/l

Na and Ca content - 400-800 mg/l

Sulfate - 20 mg/l

Identified major constituents and order-of-magnitude concentrations, mg/l

Butanol	200 mg/l
Ethanol	200
Acetic Acid	2500
Butyric acid	300
Acetaldehyde	700
Crotonaldehyde	1500

Waste D - Surfactant-Containing Waste

Total Carbon - 800-1500 mg/l

Na and Ca content - 1000-2000 mg/l

Sulfate - 55 mg/l

Identified major constituents and order-of-magnitude concentrations

Isobutanol - 60 mg/l	Crotonaldehyde- 10
Ethanol - 5	Acetone - 5
Acetic acid - 10 mg/l	Benzene - 50
Acrylic acid - 10 mg/l	Methyl ethyl pyridine - 10
Acetaldehyde - 10	Isobutyl acrylate - 20
	Surfactant concentration - 10-15 mg/l

FIGURE 34

PERFORMANCE OF ANAEROBIC PACKED COLUMN
TREATING INHIBITORY WASTEWATER
UNIT C

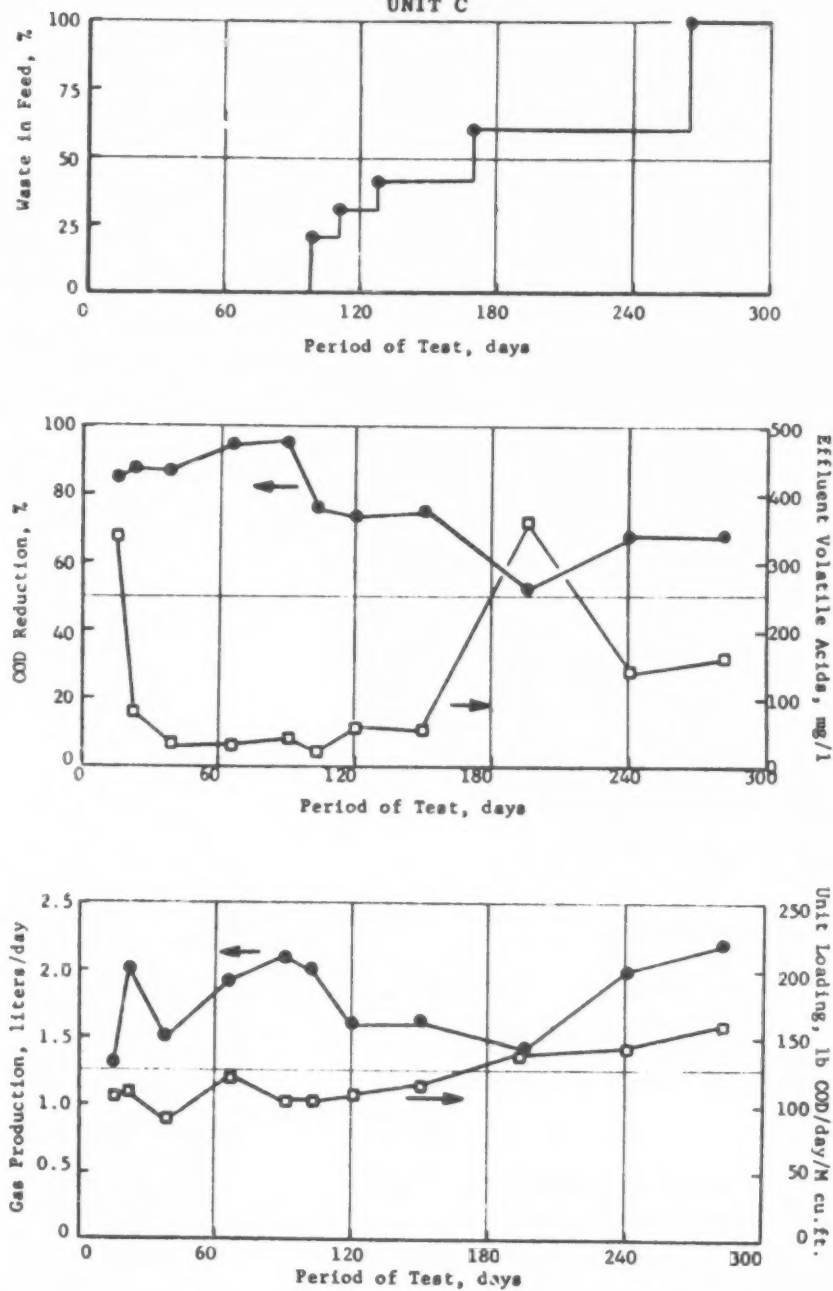


TABLE 14
DATA ON PACKED-BED ANAEROBIC REACTOR TREATING INHIBITORY WASTEWATER
(WASTE C)

Phase of Study and Significant Details of Operation	Period of Study, Days	Operational and Performance Data						
		Unit Loading, lb COD/day/M cu ft	Total Gas Production, L/day	Influent COD, mg/l	Reactor Effluent			Organic Reduction, % (COD basis)
					pH	Alkalinity, mg/l CaCO ₃	Volatiles, mg/l as Acetic	
Unit was charged with 3 liters of domestic digested sludge (~10% solids). The basic synthetic feed was employed.	1-17	108	1.3	2530	7.7	560	335	85
	18-29	112	2.0	2690	7.8	560	78	88
	30-50	90	1.5	2240	7.4	564	32	87
	51-78	117	1.9	2950	7.2	518	30	93
	79-100	102	2.1	2560	7.3	590	40	95
Started gradual introduction of diluted process wastewater (20% of the feed during this period).	101-113	101	2.0	2530	7.7	636	26	76
	114-127	107	1.6	2680	7.5	622	56	74
	128-167	114	1.6	2740	7.3	616	57	75
	168-215	138	1.4	3320	7.2	709	357	53
	216-265	144	2.0	3630	7.7	940	144	68
Diluted process wastewater 100% of unit feed.	266-297	161	2.2	4050	7.1	941	162	68

tion of the column effluent on Day 227 when the reactor was receiving 60 percent inhibitory feed indicated that the reduction in COD efficiency was caused by leakage of acetaldehyde contained in the waste and methyl isobutyl ketone contained in the supplemental feed.

Unit D - Data obtained while treating the surfactant-containing waste are presented in Table 15 and Figure 35. The waste was treated fairly well in terms of COD removal at 100 percent of the feed, although a decrease in COD removal from greater than 90 percent to 65 percent was noted above 80 percent (by volume) actual wastewater in the feed. Treatment of this unit waste was of particular interest due to contained "hard" surfactants which caused foaming problems and are not degraded in aerobic systems. If not degraded, these surfactants could be expected to cause similar problems in mixed anaerobic systems, particularly in a system employing organism separation and recycle. Surfactant measurements (cobalt-thiocyanate-active-surfactant measurements, Appendix I) indicated that the "hard" surfactants were not detected in the effluent from the anaerobic reactor at waste concentrations of 45 mg/l and below. The analysis is a measurement of surfactant remaining in its original form, since a color complex is formed with oxide chains of six or more units. Supplementary foaming experiments (Appendix I) indicated that the removal of greater than 90 percent surfactant level was accompanied by a decrease in foamability of at least 50 percent. Foamability data would be conservative since many biological systems have a tendency to produce foam.

Supplemental surfactant added to the unit waste up to a concentration of 160 mg/l resulted in a breakthrough of material measurable by the cobalt-thiocyanate method as indicated in Figure 35. Once the surfactant began to appear in the effluent there was essentially no decrease in foamability.

Discussion of Acclimation Achieved

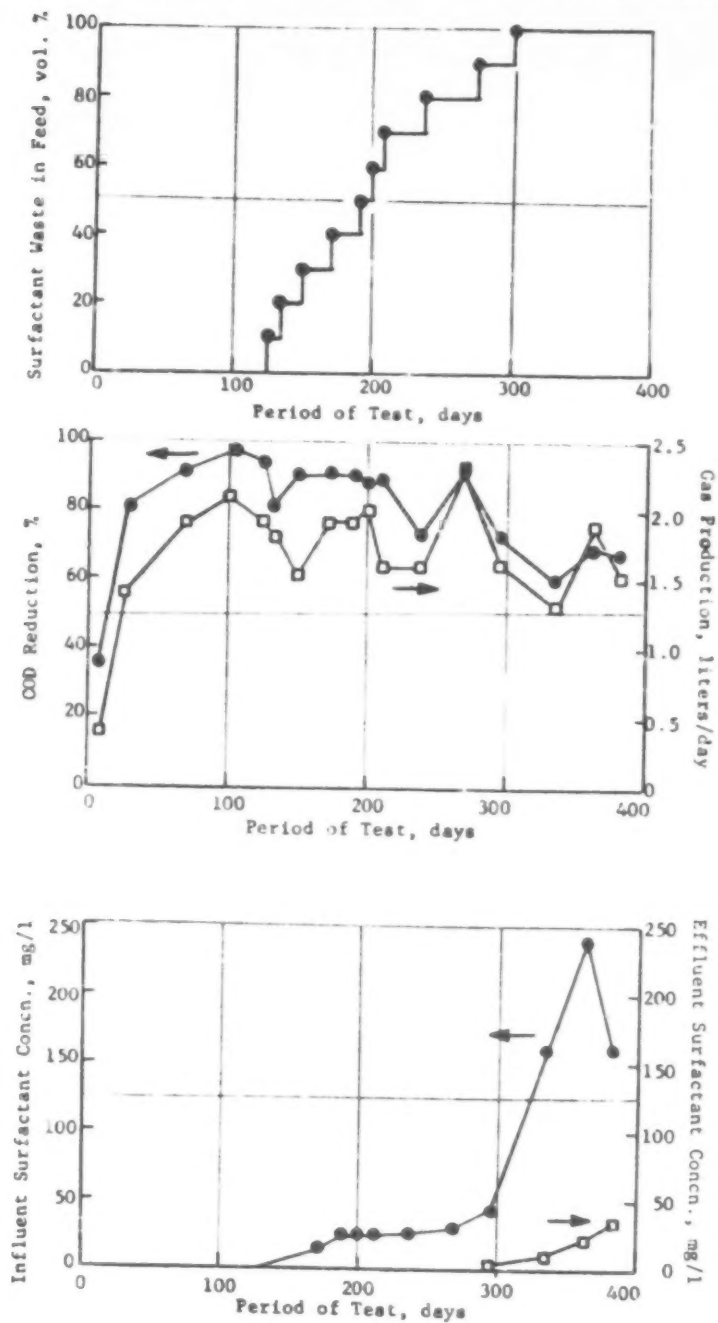
The conservative nature of the estimates of inhibitory concentrations defined during initial Warburg studies with unacclimated biomass was emphasized by the results of anaerobic bacterial acclimation studies. In many

TABLE 15
DATA ON PACKED-BED ANAEROBIC REACTOR TREATING OF SURFACTANT-CONTAINING WASTEWATER
(WASTE D)

Phase of Study and Surfactant Levels of Wastewater	% Unit Waste in Feed	Period of Study, Days	Unit Loading, lb COD/day/ft ² of reactor	Total Gas Production, ft ³ /day	Feed COD, mg/l	Feed Surfactant Conc., mg/l	Reactor Effluent				
							pH	Alkalinity, mg/l CaCO ₃	Volatile Acids, mg/l acetic	Surfactant Conc., mg/l	Organic Reduction, % (COD basis)
Charged reactor with 600 ft ³ of 3.5% (by volume) w/v synthetic food used to optimize unit performance.	0	1-12	117	0.41	2000	-	6.5	-	-	-	36
		13-43	114	1.4	2700	-	7.1	-	124	-	81
		43-91	108	1.9	2650	-	7.2	610	47	-	91
		91-121	113	2.1	2640	-	7.3	500	76	-	97
Gradual introduction of 100 mg/l wastewater	50	121-128	126	1.9	2650	5	7.7	505	11	-	94
	50	128-135	115	1.8	2650	5	7.0	509	36	-	82
	50	135-163	122	1.5	3240	10	7.7	566	50	-	90
	40	163-181	102	1.9	2750	10-20	7.3	579	36	-	91
	50	181-196	104	1.9	2700	25	7.8	750	27	-	90
	60	196-205	112	2.1	2900	25	8.2	886	41	-	88
	70	205-210	115	1.6	2880	25	7.9	895	30	-	89
	80	210-264	128	1.6	3080	25	7.7	1020	163	0	73
	93	264-276	122	2.3	2940	50	7.6	912	0	0	92
	100	276-316	115	1.6	2810	45	7.5	1005	452	1	73
		316-357	123	1.3	2940	160	7.4	1169	496	12	60
		357-389	116	1.9	2790	240	7.7	1465	277	22	69
	100	389-390	161	1.5	3880	160	7.7	1611	477	56	68

Due to plant unit
failure unit was
operated with
wastewater
from the
wastewater
treatment
plant. The
surfactant
levels were
as previously
described.

FIGURE 35
PERFORMANCE OF ANAEROBIC PACKED COLUMN
TREATING SURFACTANT CONTAINING WASTEWATER



cases compounds which were inhibitory in unacclimated Warburg studies were found treatable or able to be tolerated in considerably greater concentrations after acclimation

Successful acclimation was found to take two general courses. In the case of crotonaldehyde and ethyl acrylate the compound was found to be degraded by chromatographic analysis when acclimation was successful. It is conceivable that the compound per se could still be inhibitory but was degraded at a great enough rate so that sufficient concentrations were not reached for inhibition. In the case of phenol no decomposition of the material was observed in the anaerobic system, however, higher concentrations were tolerated after acclimation. While acclimation was successful for some single inhibitors, a more difficult problem exists with mixed inhibitor systems which may produce synergistic effects.

Acclimation was found to be unsuccessful in a digester type reactor unless complete mixing was provided. No such problem was observed in the packed bed type reactor.

Acclimation in anaerobic systems was at best a slow process. Considerable difficulty would likely be experienced in startup of a full-scale process treating an inhibitory waste.

Sulfide Studies

Inhibition due to sulfides in anaerobic treatment of petrochemical wastes was studied due to the presence of sulfate ions in many petrochemical waste streams. The sulfate ions within the stream are reduced, which produces hydrogen sulfide, that can cause toxicity (16), odor, and corrosion problems. Under the related EPA Grant 12020-DIS, "Anaerobic Treatment of Synthetic Organic Wastes," an open lagoon with a photosynthetic, sulfur-oxidizing bacterial culture was found to be effective in controlling sulfide concentrations.

In this study laboratory-scale lagoons were designed to quantify under controlled conditions of feed, temperature, and sunlight the photosynthetic

oxidation of produced sulfides. This oxidation would provide a means of off-setting toxicity of sulfides produced in anaerobic treatment of high sulfate wastes as well as yield additional capacity for reduction of oxygen-demanding wastes through re-oxidation of sulfides to sulfates and by providing an additional bacterial culture which could oxidize organic wastes. The experimental apparatus described as follows was assembled and seeded in an effort to reproduce average feed and environmental conditions observed in the previous experimental work.

Experimental Apparatus and Procedure for Sulfide Study

The laboratory lagoons consisted of two identical units as illustrated in Figure 36. The containers were constructed from glass battery jars while the tops were of plate glass, which was selected to allow passage of light wavelengths desirable to sulfur bacteria. Continuous feed of the mixed chemical feed indicated in Table 16 was provided in order to treat a representative waste and to develop an acclimated sludge. Continuous mixing was provided via a recycle pump while an incandescent-fluorescent mixed light source was connected in series with a timer to allow any light-dark ratio desired. Gas produced was measured using a wet test meter and was sampled using a hypodermic syringe.

The units were initially seeded to a depth of 3 inches with a 23 percent solids domestic primary sludge from the St. Albans, West Virginia wastewater treatment facility. Hydraulic retention time was set at 15 days. Samples from each unit were taken each working day and were analyzed daily for pH and periodically for COD, volatile acids, alkalinity, sulfides, sulfates and ORP. Daily gas production measurements were made.

Results of Sulfide Study

During the startup period both units were operated on a 12-hr, light-dark cycle. During this period, the suspended culture within the units swung from a green to a red predominant coloration. At times, one unit would be red, while the other was green. Light absorption spectra indicated the green growth had the predominant peaks for

FIGURE 36
ANAEROBIC LAGOON

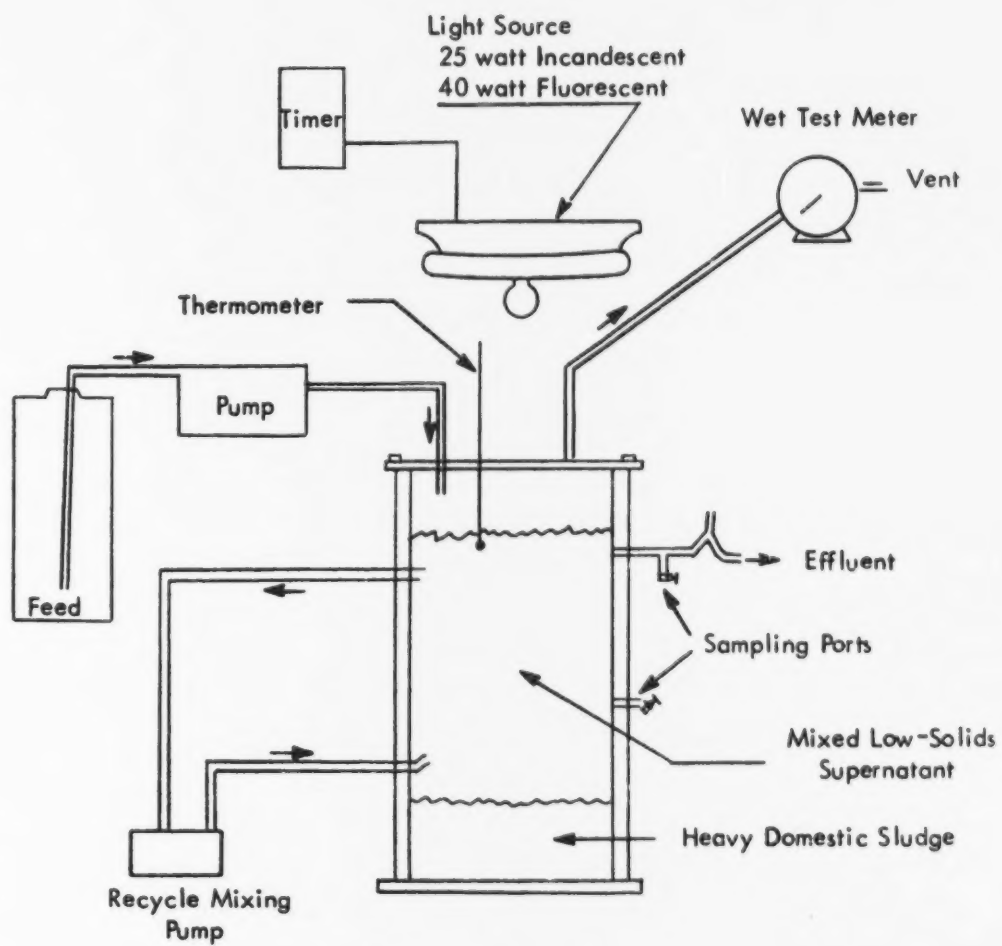


TABLE 16
ANAEROBIC LAGOON FEED

	Feed Concentration, mg/l	Equivalent TOD, ^(a) mg/l
Acetic acid	400	428
Ethanol	100	210
Ethylene glycol	200	260
Methanol	75	112
Acetone	100	220
MEK	20	50
Acetaldehyde	40	58
Sodium benzoate	20	32
Sulfate	200 (initially)	
		<u>1370</u> ^(b)

NOTE: (a) TOD based on theoretical oxidation of all carbon to CO₂ and all hydrogen to H₂O

(b) Actual COD 1230, Ratio of COD/TOD = 90%

The feed was made up as a concentrate and added to 20 liters of distilled water which was sufficient to last approximately 5 days. A more concentrated feed was made by adding more of the concentrate to the dilution water. Buffer-nutrient was described previously.

Chlorobium chlorophyll while the red growth had those characteristics of Chromatium species. Attempts were made to seed with purple sulfur bacteria from a waste treatment lagoon at Union Carbide's Texas City Plant. After approximately two months of operation the green sulfur bacteria containing Chlorobium chlorophyll became the predominant population. Attempts made to swing population predominance to purple sulfur bacteria by pH adjustment and by increasing feed stock concentration were unsuccessful, so the less common green sulfur bacteria of the family Chlorobacteriaceae were used in these studies rather than the Thiorhodaceae identified in the lagoons under Grant 12020-DIS.

In an attempt to determine whether some component in the feed was limiting growth, an experiment was designed to test growth of the red and green sulfur forms on various pure chemical substrates. Results are included in Table 17. Results are not conclusive but indicate that both green and purple sulfur bacteria can grow on the media utilized in the presence of acetic acid, ethanol, ethylene glycol, acetone and acetaldehyde. The purple sulfur bacteria were not observed to grow in the presence of methanol, methyl ethyl ketone, or sodium benzoate in this test. It is to be understood that although the photosynthetic bacteria can grow, they do not necessarily oxidize sulfides. This work was not continued due to the limited scope of the sponsoring project and lack of pure photosynthetic bacterial cultures. It could act as a starting point for other work testing inhibition of organic chemicals on lagoon ecology.

In the face of changing photosynthetic cultures the units provided approximately equal removal of applied COD during the initial period. COD removals during the length of the experiment are given in Table 18.

Experiments were also conducted during the initial period to measure diurnal changes in parameters expected to vary in a situation of bacterial photosynthesis. Measurements of pH at the end of the dark period and at the end of the light period during

TABLE 17

PHOTOSYNTHETIC BACTERIAL GROWTH ON PURE CHEMICAL SUBSTRATES

Seed	Organic Chemical ^(e)	Result ^(f)	Final pH
GSB ^(a)	Distilled water	Red (R) and Green (G)	7.6
GSB	Acetic acid	R	6.98
GSB	Ethanol	R	8.5
GSB	Ethylene glycol	R	7.3
GSB	Methanol	G	7.65
GSB	Acetone	R	7.22
GSB	Methyl ethyl ketone	White (W)	8.65
GSB	Acetaldehyde	R	7.4
GSB	Sodium benzoate	W	8.4
PSB ^(b)	Distilled water	G	8.38
PSB	Acetic acid	G, R, Brown (B)	7.0
PSB	Ethanol	G	7.38
PSB	Ethylene glycol	G & R	7.4
PSB	Methanol	G	8.02
PSB	Acetone	G & R	7.5
PSB	Methyl ethyl ketone	G	8.5
PSB	Acetaldehyde	G & R	7.8
PSB	Sodium benzoate	G	8.2
PSB ^(c)	Distilled water	G	8.0
MC ^(d)	Distilled water	G	8.0

(a) Predominately green sulfur bacteria from laboratory lagoon

(b) Predominately purple sulfur bacteria from Texas Plant lagoon

(c) Predominately purple sulfur bacteria from another Texas lagoon

(d) Mixed culture

(e) 1 ml feed stock of each named

(f) After 25 days incubation in mixed incandescent-fluorescent light

NOTE: The media used was that of Skerman for growth of *Chromatium*: Skerman, V. D. B. *Genera of Bacteria*, Williams & Wilkins Co., Baltimore, Md., 1967.

TABLE 18
SILVER LAGOON DATA

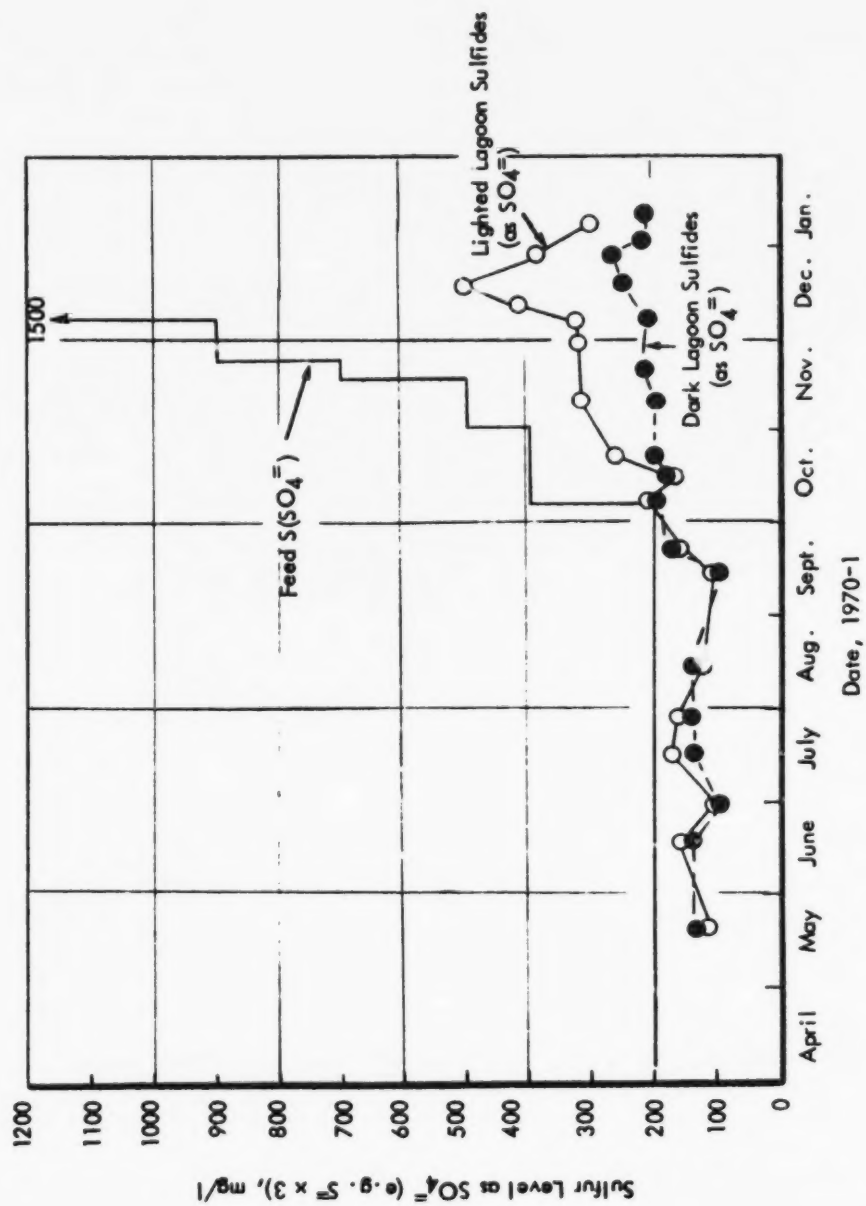
Date	Effluent COD, mg/l		COD Removal, %		SS, mg/l		SOD, %		Volatile Acids, mg/l as CH ₄		Comments
	A	B	A	B	A	B	A	B	A	B	
4/1/70	1140	260	7	38	-	-	-	-	-	-	Feed 1370 T00, 1200 COD
4/10/70	825	785	33	36	-	-	-	-	760	620	-
4/16/70	865	950	22	27	-	-	-	-	600	508	-
4/20/70	895	225	26	27	-	-	-	-	505	505	-
4/24/70	898	120	21	39	-	-	-	-	612	492	-
5/1/70	899	165	25	37	-	-	-	-	-	-	Alkalinity A 210, B 674 mg/l
5/14/70	120	805	36	24	110	129	-	-	-	-	DO = 0.0 GPD -240, -300
5/20/70	880	900	21	27	38	44	-	-	-	-	GDP -370, -280
5/25/70	470	430	85	65	-	-	-	-	-	-	-
5/29/70	535	562	56	58	-	-	-	-	-	-	-
6/3/70	750	700	39	41	-	-	-	-	487	324	-
6/8/70	880	870	28	32	-	-	-	-	-	-	40% CH ₄ 16 gms
6/12/70	880	180	47	36	-	-	-	-	32	16	-
6/17/70	718	527	41	57	26	46	37	27	-	-	-
6/18/70	685	542	44	56	56	48	39	24	-	-	0.50 AW
6/18/70	685	542	44	56	56	48	39	24	-	-	4.00 AW
6/22/70	670	635	50	47	72	46	40	24	-	-	8.30 AW
6/29/70	685	607	54	56	32	32	20	20	-	-	4.00 AW
7/2/70	805	280	50	52	-	-	22	20	-	-	Feed increased 1.5 x this date
7/7/70	970	820	47	52	-	-	22	20	561	497	GDP -370, -300
7/17/70	955	1140	48	44	51	38	-	-	545	492	Unit B covered
7/24/70	900	570	56	47	-	-	-	-	-	-	Alkalinity test A 2000, Unit B 1400
7/27/70	1405	1190	22	28	-	-	-	-	-	-	-
8/2/70	1300	1190	19	30	-	-	-	-	302	130	-
8/10/70	805	420	40	55	-	-	-	-	507	525	-
8/14/70	1100	1100	19	48	40	40	-	-	-	-	-
8/18/70	1105	1100	58	45	-	-	-	-	-	-	Feed to 3 a original this date
8/18/70	1400	1400	44	56	-	-	-	-	-	-	-
9/4/70	1782	1714	68	51	-	-	-	-	746	795	-
9/7/70	4150	1765	42	52	-	-	-	-	-	-	-
9/14/70	-	-	-	-	37	24	12	5	-	-	-
9/28/70	1405	1140	23	37	-	-	-	-	-	-	pH drop, volatile acid increase Feed off this date for 2 weeks
10/7/70	1220	1120	34	44	-	-	-	-	-	-	Feed on at 1.5 x original
10/8/70	1000	1400	29	20	-	-	-	-	-	-	2055 mg/l T00
10/14/70	1284	804	50	46	68	64	50	32	-	-	-
10/14/70	1100	805	28	48	-	-	-	-	612	460	Feed 50g, 400 mg/l
10/14/70	1275	1150	39	46	-	-	-	-	578	501	-
10/14/70	-	-	-	-	57	58	16	128	-	-	-
10/14/70	1302	1256	24	37	-	-	-	-	-	-	-
10/24/70	-	-	-	-	69	64	107	118	-	-	-
10/25/70	1318	1318	28	50	-	-	-	-	-	-	-
11/2/70	1174	1166	38	52	-	-	-	-	-	-	-
11/8/70	-	-	-	-	48	72	164	70	-	-	Feed 80g, 500 mg/l
11/12/70	-	-	-	-	84	74	116	91	-	-	-
11/16/70	800	1062	51	42	100	75	112	100	-	-	-
11/18/70	1152	1130	27	38	-	-	-	-	-	-	-
11/19/70	-	-	-	-	104	80	175	104	-	-	Feed 50g, 500 mg/l
11/24/70	-	-	-	-	68	72	195	86	-	-	-
11/24/70	1130	1010	36	47	-	-	-	-	-	-	-
11/25/70	-	-	-	-	78	56	192	86	-	-	Feed 80g, 900 mg/l 50g
11/26/70	-	-	-	-	108	68	190	24	-	-	Feed decreased to 1070 mg/l T00
12/4/70	1025	1088	16	12	-	-	-	-	-	-	-
12/4/70	-	-	-	-	108	68	210	219	-	-	-
12/8/70	1040	952	55	32	-	-	-	-	-	-	Feed 50g 1500 mg/l
12/10/70	925	-	11	-	-	-	-	-	-	-	-
12/15/70	-	-	-	-	138	50	268	528	-	-	-
12/15/70	1180	-	4	-	-	-	-	-	-	-	-
12/18/70	1487	900	23	26	82	420	530	-	-	-	Feed decreased to 450 mg/l T00
12/24/70	685	285	160	11	-	-	-	-	-	-	-
1/4/71	564	1500	28	63	-	-	-	-	-	-	-
1/5/71	-	-	-	-	100	68	1400	1620	-	-	-
1/12/71	265	150	11	63	-	-	-	-	-	-	-

one day indicated no change during the photo period (4/6 and 4/7/70). Measurements of sulfates and sulfides under the same conditions at a later date also indicated no change from day-night values (6/17/70) under the low levels of feed sulfate tested.

After essentially identical performance had been established in the two units, Unit B was covered with black polyethylene while Unit A was subjected to conditions of continuous light. The units were then operated at the initial 200 mg/l feed sulfate level while increasing the concentration of organic in the feed to determine differences in sulfur transformation in the light-dark cases under varying conditions of organic loading. As indicated in Table 18, while the feed concentration was raised to three times the original level no significant differences were observed in measured sulfide levels, also in neither unit was the sulfate concentration depleted completely as would be expected in an anaerobic environment. The increased feed brought the units almost to failure as measured by pH and volatile acid levels. Feed was, therefore, terminated for a period of two weeks and was resumed for the remainder of the experiment at a level of 1.5 times that given in Table 16.

The final state of experimentation was a rapid increase in sulfate feed concentration to unit failure. As evidenced in the plot of sulfur compounds versus time (Figure 37), the sulfates became a problem at a level between 1500 and 3000 mg/l. Sulfates were not quantitatively reduced to sulfides but lagged well behind the equivalent level of applied sulfates. During the period of initial operation approximately 75 percent of the applied sulfates were present in the mixed reactor as sulfides. Both the light and dark reactors experienced approximately the same level. The lighted system apparently maintained a greater level of bacterial activity and, hence, a more rapid conversion was evidenced during the final sulfate feed acceleration. The dark unit provided a much lower overall sulfide level. If photosynthetic oxidation were the controlling mechanism limiting sulfide level, the results would be reversed - that is, the light unit would have the lower sulfide level.

FIGURE 37
LAGOON SULFATE-SULFIDE CONCENTRATIONS



When the sulfide level within the lighted lagoon reached the 140 to 170 mg/l sulfide-ion level, the COD reduction within the unit rapidly dropped. Attempts were made to revive the system by decreasing organic feed levels with no success. The dark unit maintained a satisfactory level of reduction during this period. Strangely, the conventional parameter of pH did not forecast the unit failure as pH was satisfactory at all times. Possibly inhibition was total rather than selective for methane bacteria at the level observed. The observed level of sulfide at which inhibition occurred was slightly below the 200 mg/l soluble sulfides quoted as inhibitory by Lawrence, McCarty, and Guerin (16).

Purple sulfur bacteria reportedly (17, 18) are inhibited in their utilization of sulfides by presence of a more readily degradable feed such as acetate. The observed extent of this sparing action varies with the reporting investigator. Apparently a similar phenomena exists with the green sulfur group containing Chlorobium chlorophyll. The unit providing a light source for photosynthetic growth did not at any time exhibit significant evidence of sulfide oxidation in diurnal measurements or in comparison with an unlighted unit.

The lack of photosynthetic sulfur oxidation observed in these studies was in direct contrast to the high degree of oxidation previously reported in pilot-scale anaerobic facilities treating similar wastes (10). One immediately obvious difference between the experimental systems was the predominant bacterial groups present - Thiorhodaceae when oxidation was observed and Chlorobacteriaceae when no oxidation was observed. Some fundamental research beyond the scope of this project on environmental conditions for growth and inhibition would be required to document the cause for the change in predominance between the two systems.

SECTION VI

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SECTION VII

REFERENCES

1. Fisher, J. A., Hovious, J. C., Kumke, G. W., and Conway, R. A., Water - 1970, AIChE Chemical Engineering Process Symposium Series, 67, 107 (1971).
2. Hovious, J. C., Conway, R. A., and Harvey, Z. B., "Pilot Studies of Biological Alternatives for Petrochemical Waste Treatment," Presented at 26th Purdue Ind. Waste Conference, May 4-6, 1971.
3. Heukelekian, H., and Rand, M. C., J. Water Pollution Control Federation, 27, 9, 1040 (1955).
4. Lamb, C. R. and Jenkins, G. F., Proc. 7th Ind. Waste Conference, Purdue Univ. Ext. Ser. 79, 326 (1952).
5. Stack, V. T., Jr., Industrial and Engineering Chemistry, 49, 5, 913 (1957).
6. Hatfield, R., Industrial and Engineering Chemistry, 49, 2, 192 (1957).
7. Gerhold, R. M. and Malaney, G. W., J. Water Pollution Control Federation, 38, 4, 562 (1966).
8. Buzzell, J. C., Jr., Thompson, C. H., and Ryckman, D. W., Behavior of Organic Chemicals in the Aquatic Environment, Part III - Behavior in Aerobic Treatment Systems (Activated Sludge), Manufacturing Chemists Association (1969).
9. McCarty, P. L., Public Works, 95, 10, 123; 11, 91 (1964).
10. Hovious, J. C., Conway, R. A., and Ganze, C. W., "Anaerobic Lagoon Pretreatment of Petrochemical Wastes," Presented at 44th Annual WPCF Convention, October 3-8, 1971.
11. Umbreit, W. W., Burria, R. H., and Stauffer, J. F., Manometric Techniques, Burgess Publishing Company, Minneapolis, Minnesota, 4th ed. (1964).

12. McCarty, P. L., Jeris, J. S., and Murdock, W., J. Water Pollution Control Federation, 35, 12, 1501 (1963).
13. Lawrence, A. W. and McCarty, P. L., J. Water Pollution Control Federation, 41, R1 (1969).
14. Lank, J. C., Jr., and Wallace, A. T., "Effect of Acrylonitrile on Anaerobic Digestion of Domestic Sludge," Presented at the Purdue Industrial Waste Conference, May 1970.
15. Young, J. C. and McCarty, P. L., J. Water Pollution Control Federation, 41, 5, R160 (1969).
16. Lawrence, A. W., McCarty, P. L., and Guerin, F. J. A., Int. Journal Air Water Pollut., 10, 207 (1966).
17. Shaposhnika, V. N., Osnitskaya, L. K., and Chudina, V. I., Mikrobiologiya, 29, 9 (1960).
18. Vennes, J. W., Personal communication.
19. Standard Methods for the Examination of Water and Wastewater, 12th ed., Am. Public Health Assoc., Inc., New York (1965).
20. Mausner, M., et al., "The Status of Biodegradability Testing of Nonionic Surfactants," Scientific and Technical Report No. 6 published by the Soap and Detergent Association, October 1969.
21. Hovious, J. C., Fisher, J. A., and Conway, R. A., "Anaerobic Treatment of Synthetic Organic Wastes," Water Pollution Research Series 12020 DIS 01/72, January 1972.

SECTION VIII

APPENDIX

Analytical Methods

- A. Alkalinity - Alkalinity was determined to the methyl orange end point as given in Standard Methods (19)
- B. Anaerobic Gas Composition - Gas composition was measured using mass spectrographic equipment
- C. Chemical Oxygen Demand (COD) - COD determinations were made on filtered samples by the dichromate-reflux method as presented in Standard Methods (19)
- D. Determination of Polyether Nonionic Surfactants

Reagents

- a. Cobaltous Thiocyanate Reagent:
Dissolve 28 grams of cobaltous nitrate hexahydrate, $\text{Co}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$, and 62 grams of ammonium thiocyanate in 85 grams of distilled water. Pre-extract the reagent with three ten-ml portions of chloroform.
- b. Ethyl Ether: Reagent grade (use appropriate precautions when handling ethers)
- c. Chloroform: Reagent grade

Procedure

- a. To a 250-ml cylinder add 50 ml of sample and 50 ml of ethyl ether.
- b. Via a glass-frit sparging tube, pass nitrogen thru the aqueous phase, permitting the bubbles to form in the aqueous phase before passing into the ether layer. Allow this sparging to continue for five minutes.

- c. Pour the two phases into a separatory funnel and discard the aqueous (bottom layer).
- d. Place the separatory funnel in a steam bath and evaporate the ethyl ether (vent).
- e. After the ethyl ether is evaporated and the funnel has cooled, add 2 ml of cobaltous thiocyanate reagent.
- f. Swirl the cobaltous thiocyanate reagent over the entire surface of the funnel.
- g. Via a pipet, add five ml of chloroform to the funnel and shake for one minute.
- h. Allow the phases to separate, then draw off enough of the chloroform layer (thru a glass plug in the funnel stem) to fill a one-centimeter absorption cell.
- i. A blank is prepared by starting with step "e".
- j. Measure the absorbance of 620 m μ on a spectrophotometer (Beckman Model B or its equivalent) using the blank to set the instrument zero.

Calculation

Surfactant concentration is calculated based on standard curves prepared for the individual surfactants.

- E. Soluble Sulfides - Sulfides were monitored by Orion Research Incorporated selective ion electrode.
- F. pH - pH was measured using the Glass Electrode Method specified in Standard Methods (19).

- G. Sulfate - Sulfate ion concentrations were measured using the Turbidimetric Method presented in Standard Methods. The analysis was investigated, and no interferences were found in the anaerobic system.
- H. Volatile Acids - Volatile acid concentrations were determined by the Column-Partition Chromatographic Method presented in Standard Methods.
- I. Total Carbon (TC) - Total carbon measurements were made on filtered samples using the Union Carbide Total Carbon Analyzer.
- J. Foam - Foam measurements across the unit were made by the following procedure published by the Soap and Detergent Association (20). A 50-ml aliquot of the sample whose foaming ability was to be measured was placed in a scrupulously cleaned, 100-ml, glass-stoppered, graduated cylinder. The cylinder was shaken by hand for 15 seconds at a rate of 2 to 3 vigorous strokes in a vertical direction per second. The volume of foam was visually read from the calibrations on the cylinder after the vessel had set for 15 seconds and 60 seconds. The foam volume was read in milliliters and reported as "ml of foam per 50 ml of solution." A foam reading of zero was reserved for the condition of absolutely no foam. When the surface of the test solution was only partially covered with foam, such as by a ring of small bubbles around the periphery of the graduate, a special measuring technique was used. The volume of foam was estimated to the nearest 0.1 ml by viewing from above and comparing to a standard set of diagrams. The cylinders used were thoroughly cleaned and rinsed prior to and between foam measurements. The precision of foam measurements was improved significantly by having all determinations in a series of tests performed by the same person.

Experimental Procedures

Intermittently Mixed Digesters (Test Series #1) - The technique used in Test Series #1 utilized ten one-quart bottles as reactors, which allowed for duplicate test units for three chemicals and two biomass controls (one set of controls being fed calcium acetate). A typical bottle charge consisted of 300 ml of screened seed sludge from an anaerobic digester at a domestic wastewater treatment plant, 0.5 ml of a buffer/nutrient solution (21), 3 ml of calcium acetate solution to provide approximately 300 mg/l COD and the balance of the liter made up with tap water and test chemical dosage. Only 900 ml of this mixture were charged to the bottle as 100 ml were retained for analysis. The reactors were placed in a 35°C bath and attached to a water-displacement gas measuring system.

The reactors were manually agitated twice daily and gas production was recorded daily. Periodic unit sampling was accomplished by withdrawing 30 ml of the supernatant by attaching a 50-ml syringe to the surgical tubing on the sample tube and releasing the screw clamp. Gas readings were recorded before and after sampling in order to correct for any volume changes. Refeeding of the unit was achieved in much the same way by diluting 3 ml of acetate solution, 0.1 ml of the buffer/nutrient and the desired test chemical dosage to 30 ml in distilled water and injecting the mixture into the sample tube by syringe and then resealing the system. Although refeeding was initially set up on a regular schedule, unit activity or gas production ultimately controlled the periodic refeeding and the rate of increases in the test chemical dosages. During periods of reduced gas production, the test chemical feeding was held off to allow the system to recover.

Continuously Mixed Digesters (Test Series #2) - Subsequent acclimation test series were based on larger-volume, completely mixed reactors. Large magnetic mixers were used to supply constant agitation in the four half-gallon bottles utilized as reactors. The bottle charge consisted of the following:

995 ml tap water
500 ml screened domestic primary digester
sludge
1 ml phosphate buffer-ammonium chloride
nutrient solution (COD/N/P ratio of 100/6/2)
4.5 ml calcium acetate solution
(10 percent acetate-ion content)

The charged reactors were placed in a 35°C constant temperature bath and connected by rubber tubing to the inverted-graduate gas collection system. The water displacement bath was maintained at a pH of 4 to minimize carbon dioxide solubility. Since no pH problems were evident in the initial test series, the sampling procedure was eliminated from this study in an effort to reduce the possibility of oxygen contamination of the reactors. Refeeding procedures were the same as in the initial series, except that the volume of feed was reduced to avoid over-filling the reactor since no effluent was taken. Usually the acetate solution, test chemical, and buffer system was less than 10 ml total volume. Mixing of this feed out of the addition tube and into the reactor was obtained by pumping the syringe after the addition.

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